# Yearbook of Paediatric Endocrinology 2024

Editors
Ken Ong
Christa Flück

published by
bioscientifica

# Yearbook of
# Paediatric Endocrinology 2024

Endorsed by the European Society for Paediatric Endocrinology


**Editors**

Ken Ong
Christa Flück

**Associate Editors**

Anne-Simone Parent
Carles Gaston-Massuet
Carla Bizzarri
Cheri Deal
Diane Stafford
Evangelia Charmandari
M. Loredana Marcovecchio
Martin Wabitsch
Orit Pinhas-Hamiel
Paul van Trotsenburg
Stefano Cianfarani
Thomas Edouard
Tülay Güran

Published by Bioscientifica
Starling House, 1600 Bristol Parkway North, Bristol, BS34 8YU, UK



Typeset by Hansa Info Solutions LLP

## Editors


### Ken Ong
Medical Research Council Epidemiology Unit & Department of Paediatrics
Institute of Metabolic Science
University of Cambridge, Cambridge CB2 0QQ, UK
ken.ong@mrc-epid.cam.ac.uk

### Christa Flück
Pediatric Endocrinology, Diabetology, and Metabolism
University Children's Hospital, 3010 Bern, Switzerland
Department of Pediatrics
Department of Biomedical Research
University of Bern, 3008 Bern, Switzerland
christa.flueck@unibe.ch


## Associate Editors


### Anne-Simone Parent
Department of Pediatrics
University Hospital Liège, Belgium and
Neuroendocrinology Unit
GIGA-Neurosciences, University of Liège, Belgium
asparent@ulg.ac.be

### Carles Gaston-Massuet
Centre of Endocrinology, William Harvey Research Institute,
Barts & The London Medical School,
Queen Mary University of London, London, UK
c.gaston-massuet@qmul.ac.uk

### Carla Bizzarri
Unit of Endocrinology
Bambino Gesù Children's Hospital, Rome, Italy
carla.bizzarri@opbg.net

### Cheri Deal
Université de Montréal and Research Center, CHU Ste-Justine,
Montréal, Quebec, Canada
cheri.l.deal@umontreal.ca

### Diane Stafford
Division of Endocrinology and Diabetes
Stanford University School of Medicine
Stanford Medicine Children's Health
Palo Alto, CA 94304, USA
dejs@stanford.edu

### Evangelia Charmandari
Division of Endocrinology, Metabolism and Diabetes
First Department of Pediatrics
National and Kapodistrian University of Athens Medical School
'Aghia Sophia' Children's Hospital, and
Division of Endocrinology and Metabolism

Center of Clinical, Experimental Surgery and Translational Research
Biomedical Research Foundation of the Academy of Athens, GR- 11527,
Athens, Greece
evangelia.charmandari@gmail.com

**M. Loredana Marcovecchio**
Department of Paediatrics
University of Cambridge, Cambridge, UK
mlm45@medschl.cam.ac.uk

**Martin Wabitsch**
Division of Pediatric Endocrinology and Diabetes and Endocrine Research Laboratory
Department of Pediatrics and Adolescent Medicine
University of Ulm, Ulm, Germany
Martin.wabitsch@uniklinik-ulm.de

**Orit Pinhas-Hamiel**
Pediatric Endocrine and Diabetes Unit
Edmond and Lily Safra Children's Hospital
Sheba Medical Center, Ramat-Gan, and
Juvenile Diabetes Center,
Maccabi Health Care Services, Tel-Aviv University,
Sackler School of Medicine, IL-52621, Israel
Orit.hamiel@sheba.health.gov.il

**Paul van Trotsenburg**
Department of Pediatric Endocrinology
Amsterdam UMC
Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands
a.s.vantrotsenburg@amsterdamumc.nl

**Stefano Cianfarani**
Dipartimento Pediatrico Universitario Ospedaliero and
Department of Emergency and General Pediatrics
IRCCS "Bambino Gesù" Children's Hospital, IRCCS, 00164 Rome, Italy
Department of Systems Medicine
University of Rome Tor Vergata, Rome, Italy and
Department of Women's and Children's Health
Karolinska Institute and University Hospital, Stockholm, Sweden
stefano.cianfarani@uniroma2.it

**Thomas Edouard**
Endocrine, Bone Diseases and Genetics Unit
Reference Centre for Rare Diseases of Calcium and Phosphate Metabolism,
OSCAR Network, ERN BOND
Children's Hospital, Toulouse University Hospital
RESTORE, INSERM U1301, Paul Sabatier University; Toulouse, France
edouard.t@chu-toulouse.fr

**Tülay Güran**
Marmara University, School of Medicine
Department of Pediatric Endocrinology and Diabetes, Istanbul, Turkey
tulay.guran@marmara.edu.tr

# Table of Contents

# Preface

Welcome to the 2024 edition of the ESPE Yearbook. It has been a fascinating year of major advances both in the basic sciences and in clinical research, with several new genes, mechanisms and also highly promising treatments. Selected papers appeared in the peer reviewed literature between June 2023 and July 2024. We encourage you to explore widely the easily summarized papers and insightful comments by our expert chapter editors. And, in our ongoing quest to improve the content and format of the Yearbook, we welcome your comments and suggestions.

We are delighted to welcome as new chapter editors several leading clinical researchers in their fields: Cheri Deal (Montreal), Thomas Edouard (Toulouse), Carles Gaston-Massuet (London), Diane Stafford (Stanford), and Paul van Trotsenburg (Amsterdam). We thank retiring chapter editors, Jean-Pierre Chanoine, Ola Nilsson, Gabor Szinnai for their excellent contributions in previous years. As a new initiative this year, we are also delighted to include contributions across several chapters by members of the Young ESPE (YES) Group of young paediatric endocrinologists of the future. They were chosen by the YES Steering Committee following a call to its membership. Please join the YES Group and look out for the same call next year!

Christa E. Flück and Ken K. Ong

# 1. Pituitary and Neuroendocrinology

Zehra Yavas Abalı[1], Carles Gaston-Massuet[2]

[1]Department of Pediatric Endocrinology and Diabetes, Marmara University Pendik Research and Training Hospital, Istanbul, Turkey; [2]Centre of Endocrinology, William Harvey Research Institute, Barts & The London Medical School, Queen Mary University of London, London, UK

This year research on pituitary and neuroendocrinology has been extremely rich with outstanding contributions to the field. The number of novel mechanisms and the identification of novel disease-causing genes have increased enormously. Our selection has not been easy, and it reflects only a portion of all the excellent new findings both in basic and translational research. Highlights of this chapter include novel mechanisms with the identification of Sema6a to control vascular permeability that fine-tunes GnRH response and the role of Kisspeptin in astrocytes to control GnRH function in the HP-gonadal axis. New treatments and hopes include a successful clinical trial using MAPK inhibitors to effectively treat papillary craniopharyngiomas and the identification of novel congenital hypopituitarism-causing genes using reverse genetics from rodents to clinically relevant cohorts. Overall, the research presented in this chapter exemplifies the fast-advancing field and extraordinary basic genetic research is providing translational outputs to the benefit of the patients.

## New Mechanisms

### 1.1. SEMA6A drives GnRH neuron-dependent puberty onset by tuning median eminence vascular permeability

A. Lettieri, R. Oleari, M.H. van den Munkhof, E.Y. van Battum, M.G. Verhagen, C. Tacconi

*Nat Commun* 2023 Vol. 14 Issue 1 Pages 8097. PubMed: 38062045

Brief Summary: This research identifies a role for SEMA6A in puberty onset by regulating median eminence vascular permeability.

The authors elegantly use murine transgenics of *Sema6a-/-* combined with cell biology, biochemical assays and human genetics to identify a previously unknown role for Sema6a in regulating vascular permeability of endothelial cells within the median eminence (ME) to maintain neuroendocrine homeostasis. The role of SEMA6A in regulating the timing of puberty in humans is further demonstrated by the identification of a novel functional genetic variant, *SEMA6A* I423T in patients with delayed puberty (DP).

To support their findings, the authors first performed a thorough analysis of the embryonic and adult expression of Sema6a. Sema6a is the cell surface receptor for Plexin-A2 involved in several functions from cell migration to differentiation. In early development, Sema6a was not expressed by the GnRH neurons but instead strong expression was identified in the ME in close proximity to the axonal terminals at late gestation. Interestingly, mice lacking Sema6a exhibited normal GnRH neuronal migration but presented with a reduction of GnRH innervation at the ME. Moreover, the canonical Sema6a receptors, Plexin-A2 and Plexin-A4 were not detected in GnRH neurons, suggesting that Sema6a could function through other cell types. Analyses of $Sema6a^{-/-}$ male and female mice showed delayed puberty onset and sexual maturation defects. The number of GnRH neurons was unaffected in mice lacking Sema6a but ME innervation was reduced in both sexes.

The authors identify the source of Sema6a is the oligodendrocytes (OLs) involved in ME homeostasis. The hypothesis that Sema6a may regulate ME vascular permeability was then tested by analysing the trans-endothelial electrical resistance using in vitro cell cultures of ECs treated with extracellular Sema6a. Importantly, the authors show that Sema6a acts as a potent inducer of endothelial permeability in vitro. Sema6a null mice exhibit reduced fenestrated capillaries in the ME. Further analyses of 100 human probands with delayed puberty (DP) using exome sequencing identified a genetic variant, *SEMA6A* I423T, that leads to self-limited delayed puberty. This variant is shown to be pathogenic using in silico tools combined with cellular and biochemical assays to identify the effect of this newly DP-associated genetic variant on the development of the GnRH neuroendocrine network in humans.

In conclusion, this excellent study provides compelling evidence that SEMA6A acts as a critical regulator of puberty onset by controlling the permeability of blood vessels in the median eminence. By ensuring the proper timing of GnRH secretion, SEMA6A regulates the orderly progression of sexual maturation. This discovery not only deepens our understanding of the genetics of neuroendocrine control of puberty in humans but also opens new possibilities for therapeutic intervention in puberty-related disorders.

## 1.2. Kisspeptin signalling in astrocytes modulates the reproductive axis

E. Torres, G. Pellegrino, M. Granados-Rodriguez, A.C. Fuentes-Fayos, I. Velasco, A. Coutteau-Robles
*J Clin Invest* 2024 Jun 11;134(15):e172908
doi: 10.1172/JCI172908. PubMed:38861336

Brief Summary: This study uses a combination of proteomics and murine transgenics to identify the role of Kisspeptin signalling in astrocytes and how it impacts the reproductive axis.

Kisspeptin, a neuropeptide, is essential for regulating the hypothalamic-pituitary-gonadal (HPG) axis, which controls reproduction by acting on GnRH neurons. While most studies have focused on GnRH neurons as the primary mediators of kisspeptin signalling and function, this study highlights astrocytes, a type of glial cell in the brain, as an important modulator in this process. The researchers first identify upon stimulation of hypothalamic cells with Kisspeptin, the upregulation of astrocyte markers such as GFAP, and APP using label-free proteomics, suggesting that Kisspeptin has a function in astrocytes. They then show the expression of the Kisspeptin receptor (Kiss1r) in astrocytes and show active signalling resulting in increased phosphorylation of ERK1/2. To further elucidate the role of Kisspeptin in astrocytes, the authors elegantly delete the Kiss1r solely in the astrocytes by using an astrocyte-specific Cre line generating the G-KiR-KO murine transgenic. Male and female mice lacking Kiss1r in astrocytes display normal pubertal timing, although response to LH was increased in female mice. Deletion of Kiss1r resulted in upregulation of PGE2 which is a major upregulator of GnRH neurons. Hence, Kisspeptin action in astrocytes seems to be modulatory to fine-tune GnRH neuron's function but its depletion in astrocytes is insufficient to cause hypogonadism. The authors identify that G-KiR-KO mice under a high-fat diet (HFD) display disturbed LH pulses with lengthening of oestrus cycles indicating that Kisspeptin in astrocytes modulates the deleterious effects of HFD on the reproductive system.

In conclusion, this study shows that kisspeptin receptors on astrocytes can influence the release of gonadotropin-releasing hormone (GnRH), the key hormone initiating the reproductive cycle. This discovery adds another layer of complexity to our understanding of the reproductive axis, suggesting that astrocytes are not just passive support cells but active participants in neuroendocrine functions. The discovery of kisspeptin's interaction with astrocytes in modulating the reproductive axis challenges the long-standing neuron-centric view of reproductive GnRH regulation. These findings add to the growing body of evidence that glial cells, particularly astrocytes, are more than just supportive structures in the brain.

## 1.3. Novel candidate regulators and developmental trajectory of pituitary thyrotropes

L.Y. M. Cheung, L. Menage, K. Rizzoti, G. Hamilton, T. Dumontet, K. Basham
*Endocrinology* 2023 Vol. 164 Issue 6.
doi: 10.1210/endocr/bqad076 PubMed:37183548

Brief Summary:This study used single-cell RNA-seq and murine transgenic models combined with elegant cell lineage tracing to characterise the gene expression profiles and trajectories of pituitary thyrotropes. It identifies a novel population of pituitary thyrotropes that co-express Nr5a1 (Sf1) and Pouf1 (Pit1) and a novel developmental trajectory for a subpopulation of Nr5a1-derived thyrotropes.

Classical studies on pituitary cell lineages and cell differentiation considered that gonadotrophs and thyrotropes are derived from different transcription factors, namely Nr5a1 (Sf1, giving rise to gonadotrophs) and Pouf1 (Pit1,

thyrotropes, somatotrophs and lactotrophs). However, the transcriptional cascade that leads to thyrotropes-cell lineage specificity is not fully understood. These authors elegantly use single-cell enrichment of thyrotropes cells by using *Tshb-Cre;Rosa26-Eyfp* followed by cell sorting and single-cell RNA sequencing (scRNA-seq) to identify and characterize the gene expression profiles specific to thyrotropes. Notably, they discover new candidate genes, like Shox2 and Sox14, that play potential roles in the regulation of these cells. Shox2, for instance, was found to show significant expression in thyrotropes and appears to be linked to the transcriptional activity of the TSHβ subunit, a critical component of the thyroid-stimulating hormone. Moreover, in vivo cell lineage tracing using a Foxl2CreERT2 demonstrates the exitance of Pit1-independent dorsal lip thyrotropes.

In conclusion, this study offers significant insights into the development of pituitary thyrotropes, which are crucial to regulate thyroid function. It provides a very useful resource of thyrotropes sc-mRNA-seq data set available to the scientific community. Overall, the study highlights the complexity of pituitary thyrotrope development and opens new avenues for research into the molecular mechanisms underlying thyroid function and its associated disorders.

## 1.4. SOX9-positive pituitary stem cells differ according to their position in the gland and maintenance of their progeny depends on context

K. Rizzoti, P. Chakravarty, D. Sheridan, R. Lovell-Badge
*Sci Adv* 2023 Vol. 9 Issue 40 Pages eadf6911. PubMed:37792947

Brief Summary: This study investigated the functional diversity of SOX9-positive pituitary stem cells (PSCs) using sophisticated murine transgenics combined with single-cell mRNA-seq (sc-mRNAsq). It identifies that the ability of Sox9 + ve PSCs to maintain progeny varies depending on their location and surrounding context.

The authors elegantly show that the SOX9 transcription factor marks a population of stem cells in the pituitary gland. The authors FAC-sort Sox9 + ve cell fraction and submit these to sc-mRNA-sq. This identifies an unexpected finding that Sox9 + ve stem cells exhibit distinct characteristics based on their spatial positioning within the pituitary gland, with varying potential for differentiation and progeny maintenance. Under normal physiological conditions, these Sox9 + ve stem cells are quiescent and contribute little to new cells. However, the Sox9 + ve PSCs are mobilised and activated upon endocrine challenges by the target organ. Hence, adrenalectomy and gonadectomy trigger the activation of this population of cells. Using cell lineage tracing using a Sox9CreERT2,RosaYFP, the authors identify that the regeneration capacity and lineage commitment are dependent on the position. Importantly they show that, independent of the endocrine challenge, all endocrine pituitary cell types are initially generated, but only the required cell type survives. Hence, the local environment influences the fate of these Sox9 + ve PSCs, suggesting that stem cell behaviour is not uniform across the pituitary but rather context-dependent.

These findings highlight the importance of microenvironmental factors in regulating stem cell function. The most compelling aspect of this research is its emphasis on the context-specific nature of stem cell maintenance and progeny. By demonstrating that the surrounding microenvironment dictates how these cells behave, the study underscores the importance of studying stem cells in their native niches, rather than in isolation. The identification of which tissue environmental factors are important in mobilising PSCs could be crucial in devising regenerative therapies for pituitary disorders or endocrine diseases.

# Novel Genes

## 1.5. Imprinted Dlk1 dosage as a size determinant of the mammalian pituitary gland

V. Scagliotti, M.L. Vignola, T. Willis, M. Howard, E. Marinelli, C. Gaston-Massuet
*Elife* 2023 Vol. 12:e84092.
doi: 10.7554/eLife.84092. PubMed:37589451

Brief Summary: This study identifies the role of the *Dlk1* gene dosage in controlling the size of the pituitary gland. *Dlk1* is an imprinted gene—expression is based on parental origin—which plays a critical role in determining pituitary gland size.

The authors investigated how varying the dosage of *Dlk1* affects pituitary size using genetically modified mice. Elegantly both, loss-of-function and overexpression of *Dlk1* models were employed. Their findings suggest that *Dlk1* dosage influences the balance between stem cell self-renewal and differentiation, in a precise developmental window frame impacting on the size of the anterior pituitary. They also discovered that the effects of *Dlk1* on the pituitary are independent of overall body size, indicating a specific role in organ development rather than a general growth effect in body size.

The research has broader implications for understanding the endocrine system, particularly how imprinted genes like *Dlk1* can affect organ size and hormone production, such as growth hormone (GH). Increased *Dlk1* expression boosts stem cell replication and pituitary expansion without causing tumours, while *Dlk1* loss reduces the organ's size and GH production, pointing to the gene's importance in postnatal development and homeostasis.

This study sheds light on the molecular mechanisms behind pituitary organ size, suggesting that Dlk1's interaction with the WNT signalling pathway plays a significant role. Such insights are very important to understand pituitary homeostasis and pave the way to understanding pituitary-related diseases.

## 1.6. Knockout mice with pituitary malformations help identify human cases of hypopituitarism

J. Martinez-Mayer, M.L. Brinkmeier, S.P. O'Connell, A. Ukagwu, M.A. Marti, M. Miras
*Genome Med* 2024 Vol. 16 Issue 1 Pages 7.
doi: 10.1186/s13073-024-01347-y. PubMed:38822427

Brief Summary: This elegant study analysed a phenotype-driven screen for developmental lethal mouse genes to identify candidate genes that drive hypothalamo-pituitary phenotypes.

The authors used a publicly-available phenotype-driven screen, performed by the mouse models phenotyping facility at the DMDD Wellcome Sanger Institute, to identify key genes important in pituitary development. High-Resolution Episcopic Microscopy (HREM) from 209 knockout lines was used to identify abnormal HP-axis phenotypes and 51 candidate genes. These candidate genes were used to screen for congenital hypopituitarism (CH) genes in a large cohort of patients. 137 patients with CH and associated syndromes, previously sequenced by NGS were then analysed using a candidate gene approach from the 51 murine transgenic candidate genes. The authors identify a valuable list of new CH-candidate-causing genes and pituitary developmental-related genes.

The authors report CH-causative variants in two patients with CH in two new genes: *MORC2* and *SETD5*. *MORC2* has been previously associated with Charcot-Marie-Tooth (CMT2Z) and a developmental syndrome associated with impaired growth, craniofacial dysmorphism and axonal neuropathy (DIGFAN syndrome). *SETD5* is located on chromosome 3 and has been implicated in the 3p25 deletion syndrome characterised for severe developmental, growth restriction and delay and CNS abnormalities.

A crucial finding of this research is the identification of specific gene mutations that cause hypopituitarism. The authors use reverse genetics, starting from a murine HP-axis driven phenotypes to identify human CH-causing genes. Importantly, this study unravels important candidate genes for HP-axis development whose function is yet unknown providing a valuable resource of novel genes to study pituitary organogenesis. The study uncovered previously unrecognized genetic mutations that affect pituitary formation, and these findings were later verified in human patients with similar hormonal deficiencies. Beautifully, this article highlights a pivotal connection between mouse genetic alterations driving pituitary phenotypes and its application to identify candidate genes involved in CH and associated syndromes. Moreover, the study also brings attention to the potential for genetic heterogeneity in hypopituitarism cases. Identifying multiple gene mutations contributing to pituitary defects highlights the complexity of CH disease and the importance of comprehensive genetic analyses for accurate diagnosis and treatment planning.

## 1.7. Identification of genetic variants and phenotypic characterization of a large cohort of patients with congenital hypopituitarism and related disorders

L.C. Gregory, C. Cionna, M. Cerbone, M. T. Dattani
*Genet Med* 2023 Vol. 25 Issue 9 Pages 100881.
doi: 10.1016/j.gim.2023.10088. PubMed:37165954

Brief Summary: This study provides an in-depth exploration of the genetic underpinnings and clinical phenotypes associated with congenital hypopituitarism (CH) and related disorders. The authors analyse a large cohort (1765 patients) with or at risk of CH from 1563 unrelated families by Sanger, whole exome (WES) or whole genome sequencing (WGS). Genetic variants were identified in 10% of the CH cohort.

CH is characterized by the insufficient production of one or more pituitary hormones. It is often caused by mutations in genes involved in pituitary development and function. This study benefits from its robust sample size of 1765 patients. The authors provide a comprehensive spectrum of genetic variants associated with CH. This large dataset allows the researchers to uncover common and rare mutations, providing a detailed picture of the genetic architecture of the disease. The study goes a step further by relating genetic findings to clinical phenotypes. This phenotypic characterization is key to understanding the heterogeneity of the disease presentation, which varies widely from patient to patient. The paper provides important insights into how different genetic mutations may lead to varying degrees of hypopituitarism, from isolated hormone deficiencies to more complex syndromes involving multiple hormone deficiencies and associated developmental defects. The identification of new genetic variants in known genes also holds potential for future functional studies to understand the key genes involved in pituitary development and disease.

These findings have significant clinical implications. By correlating genetic variants with specific phenotypes, this research aids in improving diagnostic precision for congenital hypopituitarism and related disorders. Early genetic diagnosis can guide personalized treatment plans, such as hormone replacement therapy tailored to the patient's specific deficiencies. Furthermore, recognizing the genetic cause of a patient's condition can provide prognostic information and inform counselling for families regarding the likelihood of recurrence in future pregnancies.

## 1.8. The evolutionary conserved miR-137/325 tandem mediates obesity-induced hypogonadism and metabolic comorbidities by repressing hypothalamic kisspeptin

Avendaño MS, Perdices-Lopez C, Guerrero-Ruiz Y, Ruiz-Pino F, Rodriguez-Sanchez AB, Sanchez-Tapia MJ, Sobrino V, Pineda R, Barroso A, Correa-Sáez A, Lara-Chica M, Fernandez-Garcia JC, García-Redondo AB, Hernanz R, Ruiz-Cruz M, Garcia-Galiano D, Pitteloud N, Calzado MA, Briones AM, Vázquez MJ, Tena-Sempere M
*Metabolism*. 2024 Aug;157:155932.
doi: j.metabol.2024.155932. PubMed:38729600

Brief Summary: This study provides evidence for the role of miR-137/325 in obesity-induced hypogonadism (OIH) and metabolic dysfunction.

Obesity is associated with different forms of gonadal and reproductive dysfunction, including central male hypogonadism (1, 2). Although weight loss is the ideal therapeutic strategy for patients with OIH, significant weight loss is usually difficult to achieve only with lifestyle changes. Hence, androgen administration is often necessary in the management of OIH (3). Although the mechanisms involved in OIH are not well-defined, and compelling, yet fragmentary evidence has suggested the potential role of alterations of the hypothalamic Kiss1 system. It has been reported that obesity causes downregulation of receptors regulating kisspeptin neurons, which is associated with a decreased LH pulse frequency, lower LH concentrations, and hypogonadism (4).

This study investigated the mechanisms underlying OIH in males, focusing on the role of microRNAs (miRNAs) miR-137 and miR-325 in repressing hypothalamic kisspeptin expression. Various bioinformatics tools, expression and functional analyses were used to assess the impact of miR-137/325 on kisspeptin expression and the subsequent effects on reproductive and metabolic health.

The study demonstrates that miR-137 and miR-325 are upregulated in obesity, decreasing the kisspeptin expression in the hypothalamus causing low LH and testosterone concentrations. Significant improvements were achieved in both reproductive and metabolic outcomes in obese rats by reversal of this suppression. They showed that MiR-137/325 repressed human *KISS1* 3'-UTR in-vitro and inhibited hypothalamic kisspeptin content in male rats, while miR-137/325 expression was up-regulated, and Kiss1/kisspeptin decreased, in the medio-basal hypothalamus of obese rats. Selective over-expression of miR-137 in Kiss1 neurons reduced Kiss1/ kisspeptin and partially replicated reproductive and metabolic alterations of OIH in lean mice. Conversely, interference of the repressive actions of miR-137/325 selectively on Kiss1 3'-UTR *in vivo,* using target-site blockers (TSB), enhanced kisspeptin content and reversed central hypogonadism in obese rats, with the improvement of glucose intolerance, insulin resistance, and cardiovascular and inflammatory markers. Reversal of OIH by TSB miR-137/325 was more effective than chronic kisspeptin or testosterone treatments in obese rats.

In conclusion, the miR-137/325-kisspeptin pathway plays an important role in the pathogenesis of OIH and metabolic comorbidities, representing a potential therapeutic target for OIH in men.

References


1. Bombardieri A, Bufano A, Fralassi N, Ciuoli C, Benenati N, Dalmiglio C, *et al*. Assessing the Prevalence of Male Obesity-Associated Gonadal Dysfunction in Severe Obesity: A Focus on the Impact of Bariatric Surgery and Surgical Approaches. *Obes Surg.* 2024;34(9):3434-44.
2. Molina-Vega M, Muþoz-Garach A, Damas-Fuentes M, FernÃndez-GarcÚa JC, Tinahones FJ. Secondary male hypogonadism: A prevalent but overlooked comorbidity of obesity. *Asian J Androl.* 2018;20(6):531-8.
3. Shenoy MT, Mondal S, Fernandez CJ, Pappachan JM. Management of male obesity-related secondary hypogonadism: A clinical update. *World J Exp Med.* 2024;14(2):93689.
4. Villa PA, Ruggiero-Ruff RE, Jamieson BB, Campbell RE, Coss D. Obesity Alters POMC and Kisspeptin Neuron Cross Talk Leading to Reduced Luteinizing Hormone in Male Mice. *J Neurosci.* 2024;44(28).


## 1.9. Exome Sequencing has a high diagnostic rate in sporadic congenital hypopituitarism and reveals novel candidate genes


Martinez-Mayer J, Vishnopolska S, Perticarari C, Garcia LI, Hackbartt M, Martinez M, Zaiat J, Jacome-Alvarado A, Braslavsky D, Keselman A, Bergadá I, Marino R, Ramírez P, Garrido NP, Ciaccio M, Di Palma MI, Belgorosky A, Forclaz MV, Benzrihen G, D'Amato S, Cirigliano ML, Miras M, Nuñez AP, Castro L, Mallea-Gil MS, Ballarino C, Latorre-Villacorta L, Casiello AC, Hernandez C, Figueroa V, Alonso G, Morin A, Guntsche Z, Lee H, Lee E, Song Y, Marti MA, Perez-Millan MI



*J Clin Endocrinol Metab*. 2024 May 8:dgae320.
doi: 10.1210/clinem/dgae320. PubMed: 38717911


Brief Summary: This study significantly advances our understanding of the genetic underpinnings of congenital hypopituitarism (CH) by utilizing whole exome sequencing (WES) in a large cohort of patients from Argentina.

CH is a complex and highly heterogeneous disorder that is associated with highly variable clinical phenotypes that range in severity (1). The aetiology of CH may extend beyond monogenic causes, involving oligogenic, polygenic, or multifactorial factors driven by gene-environment interactions (2). High-throughput analyses offer the opportunity to identify cases of oligogenic disease, in which variants in multiple genes collaborate to produce the clinical features (3). Large cohort studies are required to detect causal variants, which may be rare or common.

This study found a diagnostic yield of 19.1% for known pathogenic (P) variants and an additional 16% for likely pathogenic (LP) variants in novel candidate genes, demonstrating the complexity and heterogeneity of the genetic aetiology of CH.

One key findings was the identification of *ROBO1* as the most frequent gene mutation in this cohort, which extends beyond its previously known association with pituitary stalk interruption syndrome (PSIS). The study also introduces new candidate genes, and members of the PTPN family, further broadening the spectrum of potential genetic causes of CH. These discoveries not only enhance our understanding of CH but also emphasize the role of WES in identifying rare and novel genetic variants that might be missed by more targeted approaches.

Moreover, the study highlights the importance of considering syndromic cases and the need for comprehensive genetic analysis, particularly in populations with diverse genetic backgrounds like Argentina. The authors advocate for the inclusion of WES (including CNV analysis) in routine diagnostic workflows for CH, especially in cases with complex or atypical presentations.

In conclusion, this study provides valuable insights into the genetic diversity of CH and reinforces the necessity of advanced genomic techniques in improving diagnostic accuracy.

References

1. Gregory LC, Cionna C, Cerbone M, Dattani MT. Identification of genetic variants and phenotypic characterization of a large cohort of patients with congenital hypopituitarism and related disorders. *Genet Med.* 2023;25(9):100881.
2. Bougnères P. Congenital Hypopituitarism: Current Agnostic Genetics Faces Its Limits. J Clin Endocrinol Metab. 2024.
3. Bando H, Urai S, Kanie K, Sasaki Y, Yamamoto M, Fukuoka H, *et al*. Novel genes and variants associated with congenital pituitary hormone deficiency in the era of next-generation sequencing. *Front Endocrinol (Lausanne).* 2022;13:1008306.

## New Treatments and Hopes

### 1.10. BRAF-MEK Inhibition in newly diagnosed papillary craniopharyngiomas

P.K. Brastianos, E. Twohy, S. Geyer, E.R. Gerstner, T.J. Kaufmann, S. Tabrizi
*N Engl J Med* 2023 Vol. 389 Issue 2 Pages 118-126.
doi: 10.1056/NEJMoa2213329. PubMed: 37437144

Brief Summary: This article reports a successful medical trial to test the therapeutic potential of targeting the BRAF-MEK pathway (using vemurafenib-cobimetinib combination therapy) in 16 patients with newly diagnosed papillary craniopharyngiomas (PCPs).

The authors recruited 16 patients with PCP who tested positive for BRAFV600E mutation, had not undergone radiation therapy and had measurable disease. The primary endpoint was objective response at 4 months using volumetric data. Importantly, 15/16 PCP patients showed a partial response with a reduction of 91% of the tumour volume.

PCP tumours, although benign, are challenging to treat due to their critical location near the hypothalamus and optic chiasm, often resulting in significant morbidity post-surgery. This study examined the efficacy of combined BRAF and MEK inhibitors (dabrafenib and cobimentinib), known to be successful in other BRAF-mutant cancers, including melanoma and thyroid cancer. It found promising results, with significant tumour shrinkage observed in patients treated with the combination therapy. The treatment modality was cycles of 28 days with patients receiving vemurafenib (960 mg orally twice daily) and cobimetinib (60 mg orally once daily) for 21 days. The primary endpoint objective response was assessed by central radiologic review of pre-specified volumetric criteria by MRI every 8 weeks. Secondary endpoints were progression-free survival, response defined by enhancing volume and adverse events. This treatment strategy represents a potential paradigm shift in managing papillary craniopharyngiomas, moving away from the traditional reliance on surgery and radiation, which often lead to long-term complications.

Clinical implications: This study indicates that targeted therapy using BRAF-MEK inhibitors may offer a less invasive and more effective option for patients with newly diagnosed PCPs, potentially preserving neurological function and improving quality of life. However, further studies are needed to assess long-term outcomes, progression of the disease after treatment and confirm the broader applicability of this treatment approach in a larger cohort of patients.

### 1.11. Central diabetes insipidus (vasopressin deficiency) after surgery for pituitary tumours: A systematic review and meta-analysis

Fountas A, Coulden A, Fernández-García S, Tsermoulas G, Allotey J, Karavitaki N
*Eur J Endocrinol*. 2024 Jul 2;191(1):S1-S13.
doi: 10.1093/ejendo/lvae084. PubMed: 38996052

Brief Summary: This comprehensive systematic review and meta-analysis provides crucial insights into the prevalence and diagnostic challenges of central diabetes insipidus (CDI), vasopressin deficiency (AVP-D), following transsphenoidal surgery (TSS) for pituitary tumours.

Despite advances in pituitary surgery techniques, posterior pituitary dysfunction remains a common and challenging complication in immediate post-operative management (1). AVP-D is the most common water balance disorder following Transsphenoidal surgery (TSS) and can be either transient or permanent, depending on the damage to the pituitary stalk or hypothalamus. Notably, many systematic reviews and meta-analyses on AVP-D prevalence after pituitary surgery lack consistent diagnostic criteria, leading to an unclear understanding of the complication's true extent (2,3).

This study aggregates data from 51 studies, including patients with pituitary adenomas, craniopharyngiomas, and Rathke's cleft cysts (RCCs), which definitively shows that AVP-D has a varied incidence across these tumour types, both transient and permanent. Transient post-operative AVP-D occurred in 17% of cases and permanent AVP-D in 3%. Permanent AVP-D occurred in 30% of craniopharyngioma surgeries, 14% of RCC cases, and only 2% of pituitary adenoma surgeries. Additionally, the prevalence rates varied depending on the diagnostic criteria used for AVP-D.

This highlights the differing effects of tumour type and surgery on hypothalamic-pituitary axis integrity. Additionally, the study emphasizes the inconsistencies in diagnostic criteria for post-operative AVP-D, leading to varied prevalence rates. The authors advocate for a standardized diagnostic approach to AVP-D, emphasizing the need for consensus on criteria such as hypotonic polyuria, hypernatremia, and desmopressin administration.

This study highlights the need for standardization in diagnosing post-operative AVP-D and emphasizes the importance of early recognition and intervention to mitigate long-term complications. Further research is warranted to refine diagnostic protocols and improve patient outcomes in this complex clinical scenario.

References


1. Hussein Z, Tzoulis P, Marcus HJ, Grieve J, Dorward N, Bouloux PM, *et al* . The management and outcome of hyponatraemia following transsphenoidal surgery: a retrospective observational study. *Acta Neurochir (Wien).* 2022;164(4):1135-44.
2. Tabaee A, Anand VK, Barrón Y, Hiltzik DH, Brown SM, Kacker A, *et al* . Endoscopic pituitary surgery: a systematic review and meta-analysis. *J Neurosurg.* 2009;111(3):545-54.
3. Chen J, Liu H, Man S, Liu G, Li Q, Zuo Q, *et al* . Endoscopic vs. Microscopic Transsphenoidal Surgery for the Treatment of Pituitary Adenoma: A Meta-Analysis. *Front Surg.* 2021;8:806855.


## 1.12. Insights into central congenital hypothyroidism: A multicenter retrospective analysis


German A, Almashanu S, de Vries L, Gil Margolis M, Halloun R, Haim A, Eyal O, Levy-Khademi F, Pivko-Levy D, Nir J, Pinhas-Hamiel O, Tenenbaum-Rakover Y



*J Clin Endocrinol Metab.* 2024 Jul 15:dgae485.

doi: 10.1210/clinem/dgae485. PubMed: 39008607


Brief Summary: This multicentre retrospective cross-sectional study provides critical insights into the epidemiology, clinical presentation, and neurodevelopmental outcomes of central congenital hypothyroidism (CCH).

CCH is a rare disorder that occurs due to insufficient hypothalamic-pituitary stimulation of the thyroid, characterized by low total T4 (TT4) with either low, normal or slightly elevated TSH. Most newborn screening (NBS) programs for CH are primarily TSH-based and thereby do not detect CCH. Only a few NBS programs worldwide aim to detect both forms of CH by different strategies. Insidious onset of the condition and the reliance on TSH-based screening protocols, which often fail to identify affected infants causes a diagnostic challenge.

This study included data from 94 patients diagnosed with CCH between 1987 and 2021. It highlights significant challenges in early detection within the framework of current NBS programs. Despite established screening efforts, the median age at diagnosis was 50 days, a delay that is particularly concerning given the high

prevalence of moderate to severe hypothyroidism at diagnosis. This underscores the limitations of NBS protocols that primarily target primary CH.

The study further emphasizes the difficulties in early detection, with only 3 infants identified through the NBS program. The predominance of multiple pituitary hormone deficiencies (MPHD) in the CCH cohort, particularly the high rates of growth hormone (96%) and ACTH (73%) deficiencies, complicates clinical outcomes, resulting in neurodevelopmental sequelae in 37% of patients. Notably, despite the critical importance of early diagnosis and intervention in CCH, the study detected no differences in neurodevelopmental outcomes between early and late-diagnosed groups. CCH prevalence was approximately 1:42,842 live births in the study and compared with the other populations and NBS strategies.

This study underscores the challenges in diagnosing and managing CCH and highlights the need for further research to assess the impact of delayed diagnosis on neurologic outcomes in affected newborns.

## 1.13. Long-term weight gain in children with craniopharyngioma

Rovani S, Butler V, Samara-Boustani D, Pinto G, Gonzalez-Briceno L, Nguyen Quoc A, Vermillac G, Stoupa A, Besançon A, Beltrand J, Thalassinos C, Flechtner I, Dassa Y, Viaud M, Arrom-Branas MB, Boddaert N, Puget S, Blauwblomme T, Alapetite C, Bolle S, Doz F, Grill J, Dufour C, Bourdeaut F, Abbou S, Guerrini-Rousseau L, Leruste A, Beccaria K, Polak M, Kariyawasam D
*Eur J Endocrinol*. 2024 May 2;190(5):363-373.
doi: 10.1093/ejendo/lvae044. PubMed: 38662730

Brief Summary: This single-centre retrospective cohort study offers valuable insights into the trajectory of weight gain in a paediatric craniopharyngioma cohort over a mean follow-up period of 10.4 years, reinforcing the necessity for targeted interventions to address this issue.

Craniopharyngioma poses a substantial clinical challenge in paediatric patients, primarily due to the risk of hypothalamic involvement. A particularly severe long-term consequence of treatment is hypothalamic obesity, characterized by excessive weight gain resulting from reduced basal metabolic rate and energy expenditure rather than increased energy intake. As a result, recent approaches have increasingly focused on hypothalamus-sparing treatments to prevent hypothalamic syndrome.

This study analysed data from 108 paediatric craniopharyngioma cases. It highlights that despite advances in hypothalamus-sparing surgical techniques, weight gain remains a major concern. Hypothalamic integrity plays a crucial role in controlling weight gain; however, even in cases where the hypothalamus was not involved, significant weight gain was still observed. This underscores the intrinsic challenge of managing hypothalamic obesity, which is less responsive to conventional lifestyle interventions due to the complex neuroendocrine dysregulation following hypothalamic damage.

Female sex, higher baseline BMI, and hypothalamic involvement by the tumour were key risk factors for greater BMI-SDS changes over time. This suggests that early intervention strategies should be personalised, particularly for high-risk groups, such as females and those presenting with a higher BMI at diagnosis. The observation that hypothalamic-sparing surgery, while beneficial, does not completely prevent weight gain, calls for ongoing exploration of adjunct therapies that target the metabolic disturbances related to hypothalamic obesity. The study also points to the need for novel therapeutic approaches.

This study underscores the need for long-term monitoring and a multidisciplinary strategy in managing paediatric craniopharyngioma, as hypothalamic obesity continues to be a significant challenge despite advances in surgical techniques

# 2. Antenatal and Neonatal Endocrinology

Cheri Deal[1], Muzna Arif[2]

[1]Université de Montréal and Research Center, CHU Ste-Justine, Montréal, Quebec, Canada; [2]Department of Paediatrics and Child Health, The Agha Khan University Hospital, Karachi, Pakistan

## New Therapies

### 2.1 Dasiglucagon for the Treatment of Congenital Hyperinsulinism: A Randomized Phase 3 Trial in Infants and Children

Thornton P, De Leon D, Empting S, Zangen D, Kendall D, Sune Birch S, Bøge E, Ivkovic J, Banerjee I

*J Clin Endocrinol Metab*. 2024;109:1071-1079.

doi: 10.1210/clinem/dgad648

Brief Summary: This open-label, randomised phase 3 trial investigated the efficacy and safety of subcutaneous infusions of Dasiglucagon[1], a glucagon analogue, as an add-on to standard of care (SoC) treatment in infants and children with congenital hyperinsulinism (CHI).

Patients had documented CHI aged 0.6 to 10.9y, who had $\geq 3$ episodes of hypoglycemia/week, defined as self-measured plasma glucose (SMPG) $<3.9$ mmol/L. Part 1 (weeks 1-4) randomised 32 children to receive either Dasiglucagon (step-up protocol, 10 to max 70 mcg/h; mean 30-35 mcg/) combined with SoC or SoC alone, and the primary outcome was (average number of SMPG values $<3.9$ mmol/L). During Part 2 (weeks 4-8) all chilren received SoC + Dasiglucagon. Demographic and baseline characteristics were comparable, with a mean age of 4.3 y (SD 2.8) and a balanced gender distribution; 66% had a gastrostomy and 34% had gone to surgery.

Dasiglucagon did not meet the primary endpoint (mean difference: 0.85 episodes per week, $P=0.5$). However, patients on Dasiglucagon had a 25% more weekly SMPG readings, suggesting more active self-monitoring. Post hoc analyses revealed a 43% reduction in CGM-detected hypoglycemia episodes on Dasiglucagon compared to SoC alone, and mean total gCHO intake was lower or decreased in children receiving Dasiglucagon (in Part 1 or Part 2).

More treatment-emergent adverse events (TEAEs) were reported on Dasiglucagon, most commonly infections, gastrointestinal disorders, and skin-related issues. Notably, hyperglycemia was reported in one patient, leading to the premature discontinuation of Dasiglucagon. Six serious, non-treatment-related events were reported.

Many drugs used to treat CHI do not have formal approval, and/or have limited effectiveness depending on CHI etiology and/or have concerning side effects. While the primary endpoint was not met, the findings provide valuable insights into the management of CHI and benefits for patient engagement. Dasiglucagon may not be sufficient as a standalone treatment, but it did allow for significant reductions in gCHO intake. In conclusion, the Dasiglucagon trial represents a significant step forward in understanding the treatment landscape for CHI.

References

1. Hawkes C, De Leon D, Rickels M. Novel Preparations of Glucagon for the Prevention and Treatment of Hypoglycemia. *Curr Diab Rep*. 2020; 19: 97. doi: 10.1007/s11892-019-1216-4.

## Congenital Hypothyroidism Outcomes

### 2.2. The longitudinal growth trajectory of children with congenital hypothyroidism during the first 3 years of life

Alinia T, Hovsepian S, Rais H, Ahmadi H, Hashemipour M

*European Journal of Pediatrics*. Epub 2024 Jul 10; 183:4123-4131.

doi: 10.1007/s00431-024-05665-6

Brief Summary: This cohort study examined the longitudinal growth trajectory of children with congenital hypothyroidism (CH) during the first 3 years of life. It involved 1474 children in Isfahan Province, Iran, and analyzed data from 2002-2022 since the initiation of CH screening (May 2002).

Overall, 38.8% of children with CH were born of consanguineous marriages and 61.6% were delivered by cesarian section, one of the highest rates in the world[1,2]. Delays were noted in weight gain (37%), linear growth (36.6%), and head growth (25.7%). The sex distribution showed a consistently higher proportion of male infants. This contrasts with the usual reported higher prevalence in females compared to males (1.5 to 2.0 to 1)[4]. Factors influencing growth patterns included gender (length/height z-score), treatment initiation age (all growth parameters), delivery method (length/height z-score), parental consanguinity (all growth parameters), history of familial hypothyroidism (all growth parameters), and thyroid-stimulating hormone (TSH) levels at 3-7 days (all growth parameters).

Three distinct trajectories of weight and head circumference z-scores were identified. An ‘’optimal growth” group (62.5% of infants) showed lower average TSH levels during the period of study. Four trajectories were noted for linear growth; the “optimal growth” group (63.4% of infants) had lower TSH on screening (mean 10.3 mIU/L) but more likely had delayed treatment initiation, suggesting that some of them had transient or less severe CH, e.g. as in pseudohypoparathyroidism. Two other groups showed declining length/height z-scores: those with the steepest decline in growth velocity (11% of infants) had the highest rate of consanguinity and highest TSH on screening (mean 50.1 mIU/L) despite timely initiation of treatment.

Barriers to achieving proper growth in children with CH include delayed diagnosis, insufficient dosage of thyroid hormone and non-adherence, and also wider factors such as poor nutrition, chronic illness, exposure to toxins, socioeconomic factors and genetic background. National studies such as this one provide valuable insights into the local prevalence (several fold higher in some areas of Iran than other countries[3,4]) and specific challenges faced in managing CH, including issues related to screening and follow-up care[5].

While many previous cross sectional and longitudinal studies have shown that the growth of children with CH is comparable to that of healthy children, a value of the current approach to identify differing growth trajectories could unmask specific groups needing more attention.

References


1. Betran A, Ye J, Moller A-B, Souza J, Zhang J. Trends and projections of caesarean section rates: global and regional estimates. *BMJ Global Health*, 2021;6:e005671. doi:10.1136/bmjgh-2021-005671.
2. Azami-Aghdash, S, Ghojazadeh M, Delhdilani N, Mohammadi M, Asa Amin Abad R. *Iranian J Publ Health*, 2014;43:545-555. PMID: 26060756 PMCID: *PMC4449402*.
3. Danner E, Niuro L, Huopio H, Niinikoski H, Viikari L, Kero J, Jääskeläinen J. Incidence of primary congenital hypothyroidism over 24 years in Finland. *Pediatric Research*, 2023:93:649–653. doi: 10.1038/s41390-022-02118-4.
4. Hemati Z, Hashemipour M, Hovsepian S, Mansourian M, Zandieh M, Ahmadian M, Dalvi M, Arefnia S, Kelishadi R. Congenital hypothyroidism in different cities of the Isfahan province: a descriptive retrospective study. *J Educ Health Promot*, 2019; 8:137. doi: 10.4103/jehp.jehp_219_18.
5. Yarahmadi S, Shahin B, Parvizi H, Motaghi R, Rahmani K. Physical development at school entry in children with congenital hypothyroidism diagnosed by the national program of newborn screening in Iran. *Int J Endocrinol Metab.* 2023; 21:e131081. doi: 10.5812/ijem-131081.


## Epigenetics

### 2.3. Trophectoderm biopsy of blastocysts following IVF and embryo culture increases epigenetic dysregulation in a mouse model


Rhon-Calderon EA, Hemphill CN, Vrooman LA, Rosier CL, Lan Y, Ord T, Coutifaris C, Mainigi M, Schultz RM, Bartolomei MS
*Human Reproduction*, 2024, 39(1), 154–176.
doi: 10.1093/humrep/dead238


Brief Summary: This study compared the histology and growth phenotypes, epigenotypes and gene expression profiles of mouse embryos, embryonic livers, and placental tissue at differing gestational ages after exposure to IVF. They followed the growth and metabolic profiles (glucose, insulin, triglycerides, total cholesterol, HDL, LDL/VLDL) of offspring to age 12 weeks. Groups analysed by sex included those conceived naturally, by IVF, by IVF with TEBx (trophectoderm biopsy) and by IVF with both TEBx and vitrification (embryo

cryopreservation). There were several differences when IVF + TEBx and IVF + TEBx + vitrification groups were compared to naturally conceived groups or those from IVF alone, with some sex dependence. TEBx/vitrification exacerbated the abnormal placental and fetal morphological and molecular phenotypes as well as adverse metabolic outcomes seen with IVF alone.

An estimated 9 million offspring have been born worldwide after assisted reproductive techniques (ART). Trophectoderm biopsy (TEBx) for preimplantation genetic testing is used to detect inherited genetic disorders and/or chromosome abnormalities in embryos. From the trophectoderm, 4-7 cells are removed for analysis. Some (but not all) studies show that TEBx may improve implantation rates and decrease miscarriages, but may not increase the number of live births particularly in younger women[1,2]. Cryopreservation through vitrification for storing retrieved embryos is another common ART procedure.

Little is known about how such procedures affect the epigenome in humans because only data on the trophoblastic (placental) epigenome is accessible. Mice provide a good model because both trophoblasts and embryos can be sampled, they do not have infertility or subfertility that confounds the human data. Although their placentation differs in some respects, both mice and humans have a discoid placenta with hemochorial gas and nutrient exchange. With their large litter sizes, it is also possible to study whether there are sex differences in the impact of TEBx +/- vitrification. As well as good animal models, all routine medical information - including details of the specific ART procedures used for conception - should be carefully recorded not only in registries but in patients' records, to allow us to better understand and monitor the impact of these procedures on health throughout the lifespan.

It should be noted that intracellular sperm injection (ICSI) was *not* used in their IVF procedures; this is another common ART method with potential consequences[3] (see the next article by Ye *et al*.).

References


1. Alteri A, Cermisoni GC, Pozzoni M, Gaeta G, Cavoretto PI, Vigan_OP. Obstetric, neonatal, and child health outcomes following embryo biopsy for preimplantation genetic testing. *Hum Reprod Update* 2023;29:291–306. doi: 10.1093/humupd/dmad001.
2. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H,Hao C *et al*. Live birth with or without preimplantation genetic testing for aneuploidy. *N Engl J Med* 2021;385:2047–2058. doi: 10.1056/NEJMoa2103613.
3. Thompson JG, McLennan HJ, Heinrich SL, Inge MP, Gardner DK, Harvey AJ. A brief history of technical developments in intracytoplasmic sperm injection (ICSI). Dedicated to the memory of J.M. Cummins. *Reprod Fertil Dev* 2024 Jun:36:RD24047. doi: 10.1071/RD24047.


## 2.4. Imprinting disorders in children conceived with assisted reproductive technology in Sweden


Ye M, Reyes Palomares A, Iwarsson E, Oberg AS, Rodriguez-Wallberg KA

*Fertility and Sterility.* 2024 Jun 1:S0015-0282(24)00517-X.

doi: 10.1016/j.fertnstert.2024.05.168. Online ahead of print



Brief Summary: This population-based national register study searched for imprinting-related disorders in all liveborn singletons in Sweden (N = 2,084,127) born between 1997 and 2017 with follow-up to Dec 2018. They identified 1,044 children with Beckwith-Wiedemann Syndrome (BWS), Prader-Willi Syndrome (PWS)/Silver-Russell Syndrome (SRS), or central precocious puberty (CPP), of whom 52 were conceived using ART therapy. The risk of being diagnosed with any of these disorders was higher among children conceived using ART therapy than in other children (Hazard ratio: 1.84; 95%CI 1.38-2.45).

This increased risk among children conceived using ART therapy was partially attenuated after adjustment for other parental factors (weighted HR 1.50; 95%CI 0.97-2.32), but persisted when restricted to children of couples with known infertility (weighted HR 1.52; 95%CI 1.05-2.01). The risk was highest for BWS (HR 2.9; 95%CI 1.86-4.53). By contrast the increased risk for PWS/SRS was non-significant (HR 1.43; 95%CI, 0.96-2.14). They also explored the impact of specific ART procedures. Compared to children born to couples with infertility and without ART, ICSI combined with frozen embryo transfer explained most of the increased risks for both PWS/SRS (weighted HR, 4.60; 95% CI, 1.72–12.28) and BWS (weighted HR, 6.69; 95% CI, 2.09–21.45). The number of cases of CPP was too low to make any valid comparisons.


While the use of assisted reproductive technology (ART) has increased steadily since the early 1980s, the first reports of its possible association with imprinting disorders in humans were published in 2002. In 2003 a meta-analyses confirmed this association[1]. Previous whole genome methylation analysis shows that more epigenetic

abnormalities are seen in cord blood cells from newborns conceived from more invasive techniques, such as ICSI + frozen embryo transfer compared to more conservative techniques of IVF with fresh embryo transfer[2,3].

Despite attempts to assess confounders in this study using subgroup analyses - not only specific parental factors but infertility per se - many other potential confounders are difficult to measure, such as potential toxins in cryoprotectants (dimethyl sulfoxide and ethylene glycol), the specific techniques used for freezing and thawing, and nutritional factors before and during early embryo and fetal development, when imprinting maintenance is essential. However, infertile couples should still be reassured that, with single embryo transfers, current IVF regimens most often result in a healthy baby. Imprinting-related disorders remain rare events.

References


1. Cortessis VK, Azadian M, Buxbaum J, Sanogo F, Song AY, Sriprasert I, Wei PC, Yu J, Chung K, Siegmund KD. Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology. *J Assist Reprod Genet.* 2018 Jun;35(6):943-952. doi: 10.1007/s10815-018-1173-x.
2. Chen W, Peng Y, Ma X, Kong S, Tan S, Wei Y, *et al*. Integrated multi-omics reveal epigenomic disturbance of assisted reproductive technologies in human offspring. *EBiomedicine* 2020;61:103076. doi: 10.1016/j.ebiom.2020.103076.
3. Liu Z, Chen W, Zhang Z, Wang J, Yang YK, Hai L, *et al*. Whole-genome methylation analysis revealed ART-specific DNA methylation pattern of neuro- and immune-system pathways in Chinese human neonates. *Front Genet* 2021;12:696840. doi: 10.3389/fgene.2021.696840.


## Laboratory Reference Values

### 2.5. Reference values for serum calcium in neonates should be established in a population of vitamin D-replete subjects


Levaillant L, Linglart A, Gajdos V, Benachi A, Souberbielle JC



*The Journal of Clinical Endocrinology & Metabolism.* 2024, Mar 13:dgae167.
doi: 10.1210/clinem/dgae167. Online ahead of print


Brief Summary: This prospective cohort study measured day 3 serum calcium and 25OH vitamin D (25OHD) in 1002 mother-newborn pairs to establish reference ranges in vitamin D replete babies.

Calcium declines in the first days of life and is frequently measured in the neonatal nursery, not only for symptomatic newborns but also for conditions such as prematurity, low birth weight or maternal diabetes mellitus. French guidelines recommend an oral bolus of Vit D3 (100,000) at the 7$^{th}$ month of pregnancy, and those not receiving or taking the prescribed dose were noted. These investigators had previously published serum total calcium levels in 1000 healthy neonates delivered at term, and measured at the same age; they published a normal range of 2.06 to 2.73 mmol/L (2.5–97.5 percentiles) – a much wider range than that seen in adults. Day 3 neonatal calcium levels in this previous study were not correlated with maternal vitamin D3 supplementation (mean ± SD with Vit D3: 2.45 ± 0.16; without Vit D3: 2.46 ± 0.16), although cord blood 25OHD was associated with day 3 neonatal calcium levels[1].

Calcium levels in both studies were measured using a colorimetric assay with an automated chemistry system (Ortho Clinical Diagnostics), and 25OH vitamin D by RIA (Diasorin, Stillwater, MN, USA) specific for 25OHD2 and 25OHD3. This study took the added step of dividing the cohort into those neonates with a serum 25OHD ≥ 30 nmol/L and those neonates with a serum 25OHD ≥ 50 nmol/L, and they excluded extreme outliers at both ends of the distributions defined by concentrations below quartile 1–1.5 x the interquartile range and above quartile 3 + 1.5 x interquartile range. This is a robust method to avoid skewing of data by outliers. This entailed removing 39 subjects from the total cohort (6 high, 33 low).

Mean ± SD (interquartile range) day 3 serum calcium was 2.46 ± 0.13 mmol/L (0.19) in the entire cohort (N = 963), and 2.50 ± 0.13 (0.18) in neonates with 25OHD ≥ 50 nmol/L (n = 208), the latter by the which the authors considered as a normal 25OHD level (although others argue ideally it should be ≥ 75 nmol/L)[2]. The normal range was 2.25–2.75 mmol/L (2.5–97.5 percentile), thus increasing the lower limit significantly.

This study reminds us that normal maternal vitamin D status is essential to maintain newborn calcium levels. Recent Endocrine Society guidelines[3] suggest that empirical supplementation during pregnancy is desirable to ensure vitamin D sufficiency, and has potential to lower risks of preeclampsia, intra-uterine mortality, preterm birth, small-for-gestational-age (SGA) birth, neonatal hypocalcemic seizures and neonatal mortality. Supplement doses and timing vary widely worldwide. In previous trials, vitamin D doses ranged from 600-5000 IU equivalent (15-125 µg) daily, given daily or weekly, or sometimes as a single large bolus as in France.

Well defined neonatal calcium ranges should also help identify neonates who need additional investigations to detect unusual disorders (such as ionised calcium, serum phosphate, magnesium, intact PTH, 25OHD and possibly 1,25OHD, with urine calcium and creatinine) particularly in newborns without predisposing factors, such as prematurity, SGA, neonatal asphyxia and the maternal factors above.


References

1. Levaillant L, Linglart A, Letamendia E, Boithias C, Ouaras-Lounis S, Thérond P, Lambert AS, Levaillant M, Souberbielle JC, Benachi A, Gajdos V. Serum calcium normal range in 1,000 term newborns. *Horm Res Paediatr.* Published online March 12, 2024. doi: 10.1159/000534042.
2. Roizen JD, Shah V, Levine MA, Carlow DC. Determination of reference intervals for serum total calcium in the vitamin D-replete pediatric population. *J Clin Endocrinol Metab*. 2013;98(12): E1946-E1950. doi: 10.1210/jc.2013-3105.
3. Demay MB, Pittas AG, Bikle DD, Diab DL, Kiely ME, Lazaretti-Castro M, Lips P, Mitchell DM, Murad MH, Powers S, Rao SD, Scragg R, Tayek JA, Valent AM, Walsh JME, McCartney CR. Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2024 Jul 12;109(8):1907-1947. doi: 10.1210/clinem/dgae290.


## Systematic Reviews

## 2.6 Safety of Antenatal Predniso(lo)ne and Dexamethasone on Fetal, Neonatal and Childhood Outcomes: A Systematic Review


Slob EMA, Termote JUM, Nijkamp JW, van der Kamp HJ, van den Akker ELT



*The Journal of Clinical Endocrinology & Metabolism.* 2024, 109, e1328–e1335.
https://doi.org/10.1210/clinem/dgad547


Brief Summary: This systematic review examined the impact of at least one trimester of pregnancy glucocorticoid treatment on pregnancy, neonatal and child outcomes. It included human studies published after 2000 (to 2022) to avoid earlier work where maternal and newborn care may have been outdated. It identified 23 eligible papers, most described cohort studies (not all had control groups), 3 case reports or series and one meta-analysis of dexamethasone (DEX) to prevent virilisation in CAH[1]. The glucocorticoids of interest were predniso(lo)ne (10 studies), DEX (11 studies) or both (2 studies).

Antenatal prednisone up to 60 mg/d and prednisolone up to 40 mg/d did not increase risk of miscarriage, stillbirth or neonatal death, there was no evidence of harmful effects on congenital abnormalities, birth weight, blood pressure, hypoglycemia or hypocortisolemia. In children followed until age 7y, (methyl)predniso(lo)ne was not associated with altered body composition, bone mass, or BMI. These somewhat reassuring results for pregnant women treated for a wide number of conditions (such as anti-SSA/Ro antibodies, rheumatoid arthritis, inflammatory bowel disease, lupus). Although monitoring of offspring is still short term, it must be remembered that these synthetic glucocorticoids can be inactivated by placental 1β-hydroxysteroid dehydrogenase type 2, unlike DEX.

For antenatal DEX, the commonly used dose 20 mcg/kg/day showed no association with lower birth weight, preterm birth or altered childhood body composition. There was a higher incidence of miscarriages and stillbirths with DEX $\geq$ 4 mg/d in early pregnancy, but it is difficult to infer causality due to prescription indication bias. Mild adrenal insufficiency was reported in 2 newborns. In longer term follow-up, antenatal DEX (1-1.5 mg/d) was associated with lower insulin secretion, and at ages $\geq$ 16y, higher plasma glucose, total cholesterol and LDL-cholesterol were noted in offspring exposed to 20 mcg/kg/d.

This systematic review concludes that girls exposed to antenatal DEX 20 mcg/kg/d had lower scores of both verbal and non-verbal intelligence, as well as verbal working memory tasks at age 7-17y. Visual spatial working memory was also negatively affected. Another excellent but non-systematic review summarised CAH diagnosis and treatment in animal and human studies with prenatal DEX exposure, including cognitive and behavioral effects[2]. Clearly more longitudinal studies are needed. Until then, updated Endocrine Society guidelines recommend antenatal DEX only for CAH and only within approved studies, and when possible, to use cell-free fetal DNA testing from as early as 6-7 weeks to avoid exposure to male fetuses[3].


References

1. Fernández-Balsells MM, Muthusamy K, Smushkin G, *et al.* Prenatal dexamethasone use for the prevention of virilization in pregnancies at risk for classical congenital adrenal hyperplasia because of 21-hydroxylase (CYP21A2) deficiency: a systematic review and meta-analyses. *Clin Endocrinol (Oxf).* 2010;73(4):436-444. doi: 10.1111/j.1365-2265.2010.03826.x.

2. Claahsen-van der Grinten HL, Speiser PW, Ahmed SF, *et al.* Congenital adrenal hyperplasia-current insights in pathophysiology diagnostics management. *Endocr Rev*. 2022;43(1):91-159. doi: 10.1210/endrev/bnab016.
3. Speiser PW, Arlt W, Auchus RJ, *et al.* Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018; 103(11):4043-4088. doi: 10.1210/jc.2018-01865.

## Artificial Intelligence

### 2.7. Transforming neonatal care with artificial intelligence: challenges, ethical consideration, and opportunities

Sullivan BA, Beam K, Vesoulis ZA, Aziz KB, Husain AN, Knake LA, Moreira AG, Hooven TA, Weiss EM, Carr NR, El-Ferzli GT, Patel RM, Simek KA, Hernandez AJ, Barry JS, McAdams RM
*J Perinatol* 2024 Jan;44(1):1-11.
doi: 10.1038/s41372-023-01848-5

Brief Summary: This paper discusses the potential use of artificial intelligence (AI) in neonatology for clinical practice and research. The authors highlight the importance of multi-stakeholder involvement, and the need for well designed protocols to not only test outcomes but also to address the ethical issues involved and usability, bias, transparency and acceptability.

We have been using AI since the 1950s when automated ECG interpretations became available using computer algorithms. Machine learning (ML) has taken AI further, using algorithms that iteratively learn from patterns in large datasets. It can be done either using labeled datasets (supervised ML) or unsupervised, which may uncover unexpected data patterns. At time of writing, a PubMed search on use of AI in healthcare reveals 20,080 publications, half of these appearing since 2022.

According to the authors, neonatology can benefit from AI due to the large wealth of clinical data generated for each infant, many of whom have complex conditions and multiple comorbidities. By linking data from clinical examination and fetal monitors with laboratory and imaging results (as well as genetic and proteomic data), prediction models can be tested for significant comorbidities, such as necrotizing enterocolitis and sepsis. AI may also help to address care of bronchopulmonary dysplasia in extreme premature and SGA babies, such as finding novel risk factors, assessing treatment strategies and predicting long-term disease burden.

Image analysis is another potential domain for AI. Retinopathy of prematurity typically requires detailed manual retinal examinations. Interpretation of retinal images by AI could decrease inter-observer variability and improve diagnostic accuracy and efficiency. Similarly, MRI imaging is often performed at term to assess brain injury in neonates with previous abnormal transcranial ultrasounds or significant comorbidities, but it has low predictive value. AI studies are examining use of computer-generated algorithms to predict both short and long term clinical outcomes.

There are multiple challenges to implementation of AI in neonatology. These include data acquisition (unbalanced or incomplete data), data processing (anomaly detection and cleaning), and data analysis and testing. All steps require quality assurance, including accuracy and reproducibility, and also ethical use of data, transparency and avoidance of bias. Users must understand the decision-making models. Government oversight will be a *sine qua non*, but regulatory bodies will be faced with constantly evolving and complex software and hardware. Given the costs of AI implementation and healthcare in general, perhaps the biggest challenge will be avoiding inequality of access to AI and to the wealth of information that can be mined and used to improve healthcare.

## Genotype-Phenotype and SRS

### 2.8. Pathogenic sequence variant and microdeletion affecting HMGA2 in Silver–Russell syndrome: case reports and literature review

Yamoto K, Saitsu H, Ohkubo Y, Kagami M, Ogata T
*Clin Epigenetics*. 2024 Jun 5;16(1):73.
doi: 10.1186/s13148-024-01688-w

Brief Summary: Two Japanese children with Silver Russell Syndrome (SRS) are reported, one with a *de novo* pathogenic frameshift sequence variant (Case 1) and the other with a 3.4 MB *de novo* microdeletion (Case 2) in *HMGA2* (High Mobility Group AT-hook 2; OMIM *600,698). Both genetic findings meet the criteria established by the ACMG/AMP[1]. Case 1 had 5 of 6 (no body asymmetry) and Case 2 had 4 of 6 (no prominent forehead or body asymmetry) of the Netchine-Harbison criteria for SRS. A literature review suggested that patients with *HMGA2* mutations may have more severe features of SRS, compared to children with SRS due to *PLAG1* mutations, *IGF2* intragenic sequence variants or *H19/IGF2:IG-DMR* epimutations.

*HMGA2* is a transcription factor gene on chromosome 12q14.3 that increases expression of both *IGF2* and *PLAG1*. In turn, *PLAG1* enhances *IGF2* expression. *HMGA2* plays a critical role in fetal growth, and common variants have been associated with child and adult height[2]. When expressed in adult cells, *HMGA2* is an oncoprotein and is highly expressed in various human cancers, serving as a prognostic marker[3].

Their literature search revealed 24 patients with 21 different *HMGA2* intragenic sequence variants/microdeletions and 23 patients with 18 different *HMGA2* microdeletions ranging from 1.35 -10.12 Mb (group 1 and group 2 for comparisons, respectively). Group 1 tended to have more frequent SRS features, whereas cleft palate and micrognathia were more frequent in group 2 suggesting that disruption of multiple genes can attenuate the SRS phenotype. Intellectual disability is more frequent in group 2, and osteopoikilosis (hyperostotic areas near articulations) is exclusively seen in group 2.

The authors suggest a possible gene dosage effect of *HMGA2* since most are heterozygous, whereas homozygous patients are more severely affected with short stature compared to their carrier parents. The microdeletion in their patient was paternally inherited and published cases were due to maternally inherited sequence variants or maternal familial microdeletions. Therefore, it is unlikely that defective imprinting of *HMGA2* is involved. Indeed, *HMGA2* is not an imprinted human locus according to the online database Geneimprint, nor imprinted in any reported mouse models[4].

The SRS phenotype is similar but more severe in patients with *HMGA2* sequence variants than those with *PLAG1* sequence variants (N = 11). This is likely due to a greater disruption of *IGF2* expression. Both are less frequent causes of SRS than *IGF2* intragenic sequence variants (N = 14) and H19/IGF2:IG-DMR epimutations (N = 226).


References

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015 May; 17(5): 405–424. doi: 10.1038/gim.2015.30.
2. Weedon MN, Lettre G, Freathy RM, Lindgren CM, Voight BF, Perry JR, Elliott KS, Hackett R, Guiducci C, Shields B, Zeggini E, Lango H, Lyssenko V, Timpson NJ, Burtt NP, Rayner NW, Saxena R, Ardlie K, Tobias JH, Ness AR, Ring SM, Palmer CN, Morris AD, Peltonen L, Salomaa V; Diabetes Genetics Initiative; Wellcome Trust Case Control Consortium; Davey Smith G, Groop LC, Hattersley AT, McCarthy MI, Hirschhorn JN, Frayling TM. A common variant of *HMGA2* is associated with adult and childhood height in the general population. Nat Genet. 2007 Oct;39(10):1245-50. doi: 10.1038/ng2121.
3. Zhang S, Mo Q, Wang X. Oncological role of HMGA2 (Review). Int J Oncol. 2019 Oct;55(4):775-788. doi: https://doi.org/10.3892/ijo.2019.4856.
4. Geneimprint (http://www.geneimprint.com/ or http://www.geneimprint.org/), Copyright © 2024, Randy L. Jirtle, PhD.


## Important Associations with Growth

## 2.9. The associations between maternal and fetal exposure to endocrine-disrupting chemicals (EDC) and asymmetric fetal growth restriction: a prospective cohort study


Hong S, Kang BS, Kim O, Won S, Kim HS, Wie JH, Shin JE, Choi SK, Jo YS, Kim YH, Yang M, Kang H, Lee D-W, Park IY, Park JS, Ko HS



*Front. Public Health.* 2024; 12:1351786.

doi: 10.3389/fpubh.2024.1351786


Brief Summary: This prospective cohort study of 146 mother-neonate pairs determined fetal-maternal exposure to EDCs from October 2021 – October 2022 and examined their associations with fetal growth parameters. Fetal exposure to bisphenol-A (BPA) showed a linear association with asymmetric fetal growth patterns.

Their definition of fetal growth restriction (FGR) when assessed by transabdominal ultrasonography at 38.3 weeks gestational age was a fetal abdominal circumference (AC) or an estimated fetal weight $< 10^{th}$ percentile[1]. Asymmetry was defined as a head circumference (HC)/AC ratio $> 95^{th}$ percentile[2]. This type of asymmetric growth restriction is typically seen in placental insufficiency when the fetal adaptation to chronic malnutrition and hypoxia spares brain growth.

Creatinine-adjusted maternal EDC exposure was assessed at the time of delivery in maternal urine and cord blood samples. They measured BPA levels as well as monoethyl phthalates (MEPs) and perfluorooctanoic acid (PFOA) by ultra-performance liquid chromatography-tandem mass spectrometry. Multiple maternal demographic characteristics and socioeconomic status indicators were collected as well as pre-conception and pregnancy habits and medical conditions that could influence fetal growth. Median age of delivery was 38.6 weeks (IQR 38.0 -39.6) with 6.2% delivered $<$ 37 weeks. A birth weight (BW) $<$ 2500 g was seen in 6.8%, and 0.7 % had a BW $>$ 4000 g. Female to male ratio was 60:40.

Linear associations were observed between maternal urine and cord blood levels of BPA and MEP, but not PFOA. There was no difference in levels of any EDC between the FGR and non-FGR groups. Conversely, a positive linear association was observed between cord blood BPA and the AH/AC ratio both before and after adjusting for several potential confounders.

Although previous work on the association of fetal exposure to BPA and BW has been controversial, this EDC is known to impact on placental development, likely by induction of oxidative stress. BPA levels have also been associated with the antiangiogenic factor sFLT-1/PIGF, a marker of placental insufficiency. This is the first study of the impact of BPA on asymmetric fetal growth, a hallmark of placental dysfunction.

References


1. Society for Maternal-Fetal Medicine (SMFM)Martins JG, Biggio JR, Abuhamad A. Society for maternal-fetal medicine consult series# 52: diagnosis and management of fetal growth restriction:(replaces clinical guideline number 3, April 2012). *Am J Obstet Gynecol*. (2020) 223:B2–B17. doi: 10.1016/j.ajog.2020.05.010.
2. Campbell S, Thoms A. Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation. *Br J Obstet Gynaecol*. (1977) 84:165–74. doi: 10.1111/j.1471-0528.1977.tb12550.x


## 2.10 Leptin and adiposity measures from birth to later childhood: Findings from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Follow-Up Study


DeLacey S, Gurra M, Arzu J, Lowe LP, Lowe WL, Scholtens DM, Josefson JL



*Pediatr Obes*. 2024 Feb;19(2):e13087.

doi: 10.1111/ijpo.13087


Brief Summary: This study was based on the HAPO prospective birth cohort, which has previously generated several publications on maternal gestational diabetes in relation to neonatal anthropometry and childhood glucose metabolism[1,2]. It examined if cord blood leptin was positively associated with peripubertal obesity and adiposity, and if this relationship was dependent on maternal BMI, hyperglycemia and/or other maternal factors.

This is an important question, because animal models have shown that interfering with perinatal leptin exposure can impact on adiposity outcomes in older offspring[3] suggesting it could be a biomarker for both risk identification and prevention.

This secondary analysis included a highly diverse subgroup of 986 pregnant mothers, chosen from the initial 23,316 participants, recruited in 15 centers from 9 countries between 2000 and 2006. Offspring were examined within 3 days of birth and again at 10-14 y old (mean $11.5 \pm 1.1$ y). Mothers were of different ethnicities (44% White, 25% Black, 16% Asian, 14% Hispanic), and 15% had gestational diabetes. Mean gestational age at delivery was $39.9 \pm 1.9$ w, mean birthweight was $3402.7 \pm 466.6$, and half were females. At childhood follow-up, 28% were overweight or obese, and 11% obese. Cord blood leptin (log-transformed) and multiple measures of adiposity were also recorded at birth and again at follow-up, along with pubertal status.

Linear regression was performed, adjusting for several maternal covariates. As expected, cord blood leptin was positively associated with neonatal adiposity, and also with peripubertal leptin. Positive associations between

cord blood leptin and childhood obesity were partially attenuated by adjustments for maternal BMI and glucose. They remained significant for childhood % body fat, body fat mass, sum of skinfolds, and log-transformed serum leptin. The odds ratio (OR) for overweight or obesity was 1.21 (95% CI = 1.03-1.42), for obesity (OR = 1.31, 95% Cl = 1.04-1.66) and for % body fat > 85th percentile (OR = 1.38, 95% Cl = 1.12-1.73).

The robust and consistently positive relationships observed here contrast with previously reported inconsistent results. The authors hypothesize this may be due to their larger sample size, the high proportion of overweight and obese children, and older age at follow-up, as the peripubertal period appears to be a metabolic turning point. The mechanism by which neonatal leptin impacts future adiposity remains to be determined.

References


1. Lowe WL Jr, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, Catalano PM, Linder B, Brickman WJ, Clayton P, Deerochanawong C, Hamilton J, Josefson JL, Lashley M, Lawrence JM, Lebenthal Y, Ma R, Maresh M, McCance D, Tam WH, Sacks DA, Dyer AR, Metzger BE. Hyperglycemia and adverse pregnancy outcome follow-up study (HAPO FUS): maternal gestational diabetes mellitus and childhood glucose metabolism. *Diabetes Care*. 2019;42(3):372-380. doi: 10.2337/dc18-1646.
2. Group HSCR. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. *Diabetes*. 2009;58(2):453-459. doi: 10.2337/db08-1112
3. Stocker CJ, Cawthorne MA. The influence of leptin on early life proÙgramming of obesity. *Trends Biotechnol*. 2008;26(10):545-551. doi: 10.1016/j.tibtech.2008.06.004.


## 2.11 Cord Blood Proteomic Profiles, Birth Weight, and Early Life Growth Trajectories


Van Pee T, Martens DS, Alfano R, Engelen L, Sleurs H, Rasking L, Plusquin M, Nawrot TS



*JAMA Netw Open*. 2024 May 1;7(5):e2411246.
doi: 10.1001/jamanetworkopen.2024.11246


Brief Summary: This prospective singleton birth cohort (N = 288, 43.4% male) from Flanders, Belgium, was a subset of a larger longitudinal cohort (ENVIRONAGE). The authors used a targeted proteomic panel to measure 386 inflammatory-related proteins in cord blood and examined their associations with birth weight (BW), birth weight ratio (BWR - BW divided by the median BW for gestational age for sex and parity), and rapid infant weight gain (defined as [weight z score at 12 m minus BW z score] > 0.67). Other outcomes at 4-6 y were weight, body mass index (BMI), waist circumference and overweight. Multiple logistic regression models were adjusted for several maternal and offspring factors and Bonferroni correction was used to correct for multiple testing.

Mother's pre-pregnancy BMI was (mean ± SD) 24.1 ± 4.1, 52.4% were primiparous, 66.3% had a college or university diploma, gestational age was 39.6 ± 1.7 weeks, birth weight 3389 ± 493 g (10% > 4000 g; 2.4% < 2500 g) and 95% had at least 2 European grandparents. 31% showed rapid infant weight gain, and 11.8% were overweight or obese at 4-6 y.

Seven proteins were of significant interest; these were involved in GH pathway, metabolism and metabolic disorders, neurological pathways and placental vascularization. Positive associations with BWR and/or BW were seen with afamin and SFRP4. Both proteins are linked to the Wnt signaling pathway, which is involved in cell proliferation, survival migration and polarity in pre- and postnatal life. Afamin overexpression in transgenic mice leads to increased birth weight, and elevated afamin and SFRP4 concentrations in the first trimester in humans are associated with gestation diabetes.

Lower cord blood levels of 5 proteins showed negative associations with growth. EPHA4 (which has a positive role in IGF-1R signalling and postnatal body growth in mice), TCN1 (which binds vitamin B12, and B12 deficiency is associated with lower BW), CELSR2, SLITRK1, and UNC5D (these latter 3 are involved in neurological pathways but yet unknown roles in growth). For rapid infant weight gain and overweight at age 4-6 y, odds ratios (and 95% CI) were well below 1.0 for afamin, and a trend was noted for SFRP4: OR 0.49; 0.29-1.01 for rapid infant weight gain). In contrast, an elevated OR (2.44; 95% CI 1.26-4.8) was seen for TCN1 and rapid infant weight gain. Forest plots per doubling of protein levels were also calculated for weight, BMI and waist circumference at age 4-6 y and again pointed to negative associations of EPHA4, SLITRK1, CELSR2 and UNC50.

This study presents many novel findings. None of the associations appeared to be sex-specific. Further studies should explore how stress during pregnancy can influence the fetal proteome and contribute to newborn growth as well as to future disease risks.

## 2.12 Early Detection of Adrenal Insufficiency: The Impact of Newborn Screening for Adrenoleukodystrophy

Ramirez Alcantara J, Grant NR, Sethuram S, Nagy A, Becker C, Sahai I, Stanley T, Halper A, Eichler FS

*J Clin Endocrinol Metab*. 2023 Oct 18;108(11):e1306-e1315.
doi: 10.1210/clinem/dgad286

Brief Summary: This retrospective chart review included 116 patients aged 0 to 17 y (M:F 94%:6%) with X-linked adrenoleukodystrophy (ALD) managed in one expert medical center from 2006 to 2022. It assessed the impact of newborn screening (which began in the U.S. in 2013 based on measurement of a lysophosphatidylcholine derivative of a very long chain fatty acid (VLCFA) C26:0-LPC, followed if abnormal by *ABCD1* gene sequencing), on the age and presentation of adrenal insufficiency (AI) in ALD. AI was detected in 80% of males and most often before age 10 y. All patients were either on maintenance and/or stress dose glucocorticoid treatment.

Newborn screening (NBS) accounted for 31 patients (27%; M:F 26:5). Age at ALD diagnosis decreased with time, and was younger in those diagnosed by NBS (NBS vs. non-NBS diagnosis: 28% vs. 4% by age 1 y; 47% vs. 19% by age 3 y). Mean time to AI detection was shorter in patients diagnosed by NBS (3.9 y) than in those with non-NBS diagnosis (7.1 y). The overall prevalence of AI was 74%. Of these, 51% had symptomatic AI and 41% had latent AI (4% had insufficient data, all with later diagnosis). Of interest, 13 were diagnosed with AI prior to their diagnosis of ALD.

In all patients who developed AI, the median time to transition from stress to maintenance glucocorticoid treatment was 1.46 y. There was a trend to younger glucocorticoid transition in the NBS diagnosis group, likely due to current recommendations for repeated AI screening in ALD patients[1]. When maintenance glucocorticoid treatment was initiated, both ACTH and peak cortisol levels were lower in patients diagnosed by NBS, although median values were abnormal in both NBS and non-NBS diagnosis groups.

Many unknowns remain. There is insufficient understanding of the genotype-phenotype relationships. Controversy exists concerning the management of positive NBS findings in females. While women with ALD develop myelopathy (typically at an older age than men), adrenal insufficiency and cerebral ALD are fortunately very rare[2]. Finally, future studies should consider the inclusion of other forms of peroxisomal disorders in NBS. Recent data suggests that Zellweger Spectrum Disorder can be picked up in ALD screening programs; such patients also are at risk of AI but their natural history is not yet fully predictable or understood[3,4].

### References

1. Engelen M, van Ballegoij WJC, Mallack EJ, Van Haren KP, Köhler W, Salsano E, van Trotsenburg ASP, Mochel F, Sevin C, Regelmann MO, Tritos NA, Halper A, Lachmann RH, Davison J, Raymond GV, Lund TC, Orchard PJ, Kuehl JS, Lindemans CA, Caruso P, Turk BR, Moser AB, Vaz FM, Ferdinandusse S, Kemp S, Fatemi A, Eichler FS, Huffnagel IC. International Recommendations for the Diagnosis and Management of Patients With Adrenoleukodystrophy: *A Consensus-Based Approach Neurology.* 2022 Nov 22;99(21):940-951. doi: 10.1212/WNL.0000000000201374.
2. Kemp S, Huffnagel IC, Linthorst GE, Wanders RJ, Engelen M. Adrenoleukodystrophy - neuroendocrine pathogenesis and redefinition of natural history. *Nat Rev Endocrinol.* 2016 Oct;12(10):606-15. doi: 10.1038/nrendo.2016.90.
3. Mares Beltran CF, Tise CG, Barrick R, Niehaus AD, Sponberg R, Chang R, Enns GM, Abdenur JE. Newborn Screening for X-Linked Adrenoleukodystrophy (X-ALD): Biochemical, Molecular, and Clinical Characteristics of Other Genetic Conditions. *Genes (Basel).* 2024 Jun 26;15(7):838. doi: 10.3390/genes15070838.
4. Bose M, Yergeau C, D'Souza Y, Cuthbertson DD, Lopez MJ, Smolen AK, Braverman NE. Characterization of Severity in Zellweger Spectrum Disorder by Clinical Findings: A Scoping Review, Meta-Analysis and Medical Chart Review. *Cells.* 2022 Jun 10;11(12):1891. doi: 10.3390/cells11121891.

# 3. Thyroid

Paul van Trotsenburg[1], Elham Atiq[2]

[1]Department of Pediatric Endocrinology, Emma Children's Hospital Amsterdam University Medical Center, Amsterdam, The Netherlands; [2]Sultan Qaboos Hospital, Salalah, Sultanate of Oman

In the past year, there were a large number of scientific publications on disorders of the thyroid gland or the hypothalamus-pituitary-thyroid (HPT) axis in children and adults. In this chapter, you will find a selection of the probably most impactful publications in this field published in the period June 2023 to July 2024, of importance to the discipline of pediatric endocrinology.

To get you excited, here is a short overview: "Disruption of Notch signaling in mice thyrocytes causes hypothyroidism, possibly explaining hypothyroidism in Alagille syndrome", "Creation of healthy ranges of serum TSH and FT4 in adults, based on the risk of cardiovascular disease and mortality", "Ultradian TSH pulses are more efficient than sustained rise in basal TSH levels at increasing thyroid hormone production in mice", "TRH neurons in the paraventricular nucleus are major regulators of the HPT axis and the fasting-induced suppression of TH levels in mice; the latter relies, at least in part, on the activation of agouti-related protein / neuropeptide Y neurons in the arcuate nucleus of the hypothalamus", "Mutations in a noncoding $(TTTG)_4$ microsatellite/short tandem repeat located at 15q26.1 are the probable cause of a considerable percentage of cases of non-goitrous primary congenital hypothyroidism", "Identification of SLC22A9 and SLC29A2 as transporters mediating cellular uptake of 3,5,3'-Triiodothyroacetic acid (TRIAC)", and "Genotype data of 11,220 5-year childhood survivors shows that cancer-specific polygenic risk scores derived from general population genome-wide association study cancer loci, identifies survivors of European ancestry at increased risk of subsequent thyroid cancer".

## Mechanism of the Year

### 3.1. Notch signaling in thyrocytes is essential for adult thyroid function and mammalian homeostasis

Lluc Mosteiro, Thi Thu Thao Nguyen, Simona Hankeova, Daniel Alvarez-Sierra, Mike Reichelt, Shannon M Vandriel, Zijuan Lai, Feroza K Choudhury, Dewakar Sangaraju, Binita M Kamath, Alexis Scherl, Ricardo Pujol-Borrell, Robert Piskol, Christian W Siebel

*Nat Metab*. 2023 Dec;5(12):2094-2110.
doi: 10.1038/s42255-023-00937-1. PMID: 38123718

Brief summary: This study used single-cell RNA sequencing to analyze mouse and human thyroid tissue. It discovered two distinct subtypes of thyrocytes, termed thyroid follicular cell (TFC) 1 and TFC2. These subtypes were characterized by varying levels of metabolic activity and Notch signaling: TFC1 cells exhibit higher metabolic activity and Notch signaling compared to the more quiescent TFC2 cells.

Notch signaling is a crucial pathway involved in cell fate determination, and its role in thyroid function was explored using mouse models. By a combination of genetic and pharmacological approaches to disrupt Notch signaling specifically in thyrocytes, mice with targeted deletion of Notch1 and Notch2 in thyrocytes, or treated with Notch pathway-blocking antibodies, developed decreased serum thyroid hormones (TH) and increased TSH levels, and significant dysregulation of whole-body thermoregulation. Further investigation showed that Notch inhibition disturbs a thyrocyte-specific transcriptional program, and induces thyrocyte defects due to decreased mitochondrial activity and reactive oxygen species (ROS) production, which are necessary for TH production. These thyrocyte/mitochondrial defects explained the hypothyroidism observed in the Notch-inhibited mice.

Finally, the authors studied a cohort of 72 children with Alagille syndrome - a disorder caused by mutations in Notch pathway components (JAG1 or NOTCH2). Five patients (7%) were diagnosed with hypothyroidism, representing a 70-fold increase relative to the 0.1% estimated prevalence of pediatric hypothyroidism, confirming a connection between the findings in mice and thyroid dysfunction observed in Alagille syndrome [1].

In September 2023 Wu FY *et al*. and in March 2024 Zhang HY *et al*. reported pathogenic variants in *MAML2* and *MAMLD1*, and in *CNTN6*, respectively, in patients with congenital hypothyroidism due to dyshormonogenesis [2,3]. Pathogenic variants in *MAML2* and *MALD1*, as well as in *CNTN6* downregulated Notch signaling, stressing the importance of the findings and new insights about Notch signaling in thyrocytes by Monteiro *et al*.

References

1. de Filippis T, Marelli F, Nebbia G, Porazzi P, Corbetta S, Fugazzola L, Gastaldi R, Vigone MC, Biffanti R, Frizziero D, Mandarà L, Prontera P, Salerno M, Maghnie M, Tiso N, Radetti G, Weber G, Persani L. JAG1 Loss-Of-Function Variations as a Novel Predisposing Event in the Pathogenesis of Congenital Thyroid Defects. *J Clin Endocrinol Metab.* 2016 Mar;101(3):861-70. doi: 10.1210/jc.2015-3403. Epub 2016 Jan 13. PMID: 26760175
2. Zhang HY, Wu FY, Zhang CX, Wu CY, Cui RJ, Liu XY, Yang L, Zhang Y, Sun F, Cheng F, Yang RM, Song HD, Zhao SX. Contactin 6, A Novel Causative Gene for Congenital Hypothyroidism, Mediates Thyroid Hormone Biosynthesis Through Notch Signaling. *Thyroid.* 2024 Mar;34(3):324-335. doi: 10.1089/thy.2023.0594. Epub 2024 Feb 12. PMID: 38183624
3. Wu FY, Yang RM, Zhang HY, Zhan M, Tu PH, Fang Y, Zhang CX, Song SY, Dong M, Cui RJ, Liu XY, Yang L, Yan CY, Sun F, Zhang RJ, Wang Z, Liang J, Song HD, Cheng F, Zhao SX. Pathogenic variations in MAML2 and MAMLD1 contribute to congenital hypothyroidism due to dyshormonogenesis by regulating the Notch signalling pathway. *J Med Genet.* 2023 Sep;60(9):874-884. doi: 10.1136/jmg-2022-108866. Epub 2023 Mar 10. PMID: 36898841

## Hypothalamus-Pituitary-Axis Regulation

### 3.2. Regulation of thyroid hormone levels by hypothalamic thyrotropin-releasing hormone neurons

Ricardo H Costa-E-Sousa, Rodrigo Rorato, Anthony N Hollenberg, Kristen R Vella

*Thyroid*. 2023 Jul;33(7):867-876.
doi: 10.1089/thy.2023.0173. PMID: 37166378

Brief summary: This study examined the regulation of thyroid hormone (TH) levels by thyrotropin-releasing hormone (TRH) neurons located in the paraventricular nucleus (PVN) of the hypothalamus in fed and fasted mice. It focused on the role of agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons in the arcuate nucleus.

Using chemogenetic activation in mice, the authors demonstrated that TRH neurons play a direct role in regulating the hypothalamic-pituitary-thyroid (HPT) axis. Activation of TRH neurons led to an increase in TSH and TH levels in both fed and fasted states, confirming their involvement in TH homeostasis. They then investigated the interaction between TRH neurons and AgRP/NPY neurons. Stimulation of AgRP/NPY neurons suppressed the HPT axis, despite increasing food intake. Inhibition of these neurons prevented the fall in TH levels during a fast, presumably via direct regulation of PVN TRH neurons via, in part, the melanocortin 4 receptor (MC4R). Additionally, the study finds that TRH-mediated feedback is independent of TH receptor beta (TRb) signaling in MC4R neurons, challenging previous hypotheses about the role of these neurons in TH regulation.

This study identifies TRH neurons as critical regulators of the hypothalamus-pituitary-thyroid axis and shows that fasting-induced TH suppression is mediated by NPY/AgRP neurons, and not - as was previously thought - by fasting-induced hypothalamic type 2 deiodinase expression and activity, increasing hypothalamic T3 production.

### 3.3. TSH pulses finely tune thyroid hormone release and TSH receptor transduction

Anne Guillou, Yasmine Kemkem, Chrystel Lafont, Pierre Fontanaud, Davide Calebiro, Pauline Campos, Xavier Bonnefont, Tatiana Fiordelisio-Coll, Ying Wang, Emilie Brûlé, Daniel J Bernard, Paul Le Tissier, Frederik Steyn, Patrice Mollard

*Endocrinology*. 2023 Nov 20;165(1):bqad164.
doi: 10.1210/endocr/bqad164. PMID: 37934802

Brief summary: This study examined the pulsatile secretion of TSH and its regulatory role in thyroid hormone (TH) synthesis in mice. Using a novel ultra-sensitive ELISA for mouse TSH, it shows that TSH pulsatility plays a critical role in regulating thyroid function.

In addition to mapping the normal TSH secretion pattern in healthy mice, and the secretion pattern in a mouse model of disease (mouse made hypothyroid by an iodine-deficient diet enriched with propylthiouracil), experiments show that repeated short bursts of TSH secretion (ultradian pulses) are more effective in stimulating TH hormone production than sustained increases in TSH levels. Additional experiments investigating the role of TSH in thyroid follicle signal transduction, particularly via cAMP, finds that ultradian TSH pulses produce stepwise increases in intracellular cAMP, enhancing thyroid function. In contrast, continuous TSH stimulation leads to an initial cAMP rise, which then declines over time, suggesting that thyroid cells respond better to pulsatile TSH patterns.

This study highlights the importance of TSH pulsatility in thyroid function regulation, showing that TSH pulses are more effective than sustained increases in stimulating TH production. The experimental framework developed here will not only allow studying thyroid sensing of TSH secretion patterns in relation to the kinetics of signal transduction downstream of the TSH receptor, as the authors suggest, but may also be used to gain more insight in the precise causes of decreased TH synthesis/secretion in central congenital hypothyroidism (CH), by using mouse models expressing targeted mutations in *IGSF1* or other genes associated with central CH like *TBL1X* or *IRS4* [1]. In 2016, Joustra *et al* reported that patients with central CH due to IGSF1 deficiency showed decreased pulsatile secretion of TSH with decreased disorderliness and reduced diurnal variation. This however, was investigated in only three patients [2].

References


1. Lauffer P, Zwaveling-Soonawala N, Naafs JC, Boelen A, van Trotsenburg ASP. Diagnosis and Management of Central Congenital Hypothyroidism. *Front Endocrinol (Lausanne).* 2021 Sep 9;12:686317. doi: 10.3389/fendo.2021.686317. eCollection 2021. PMID: 34566885
2. Joustra SD, Roelfsema F, Endert E, Ballieux BE, van Trotsenburg AS, Fliers E, Corssmit EP, Bernard DJ, Oostdijk W, Wit JM, Pereira AM, Biermasz NR. Pituitary Hormone Secretion Profiles in IGSF1 Deficiency Syndrome. *Neuroendocrinology.* 2016;103(3-4):408-16. doi: 10.1159/000439433. Epub 2015 Aug 25. PMID: 26336917


## Thyroid Function - Genetic Determinants and Associations with Health and (Thyroid) Disease

### 3.4. Genome-wide association study of thyroid-stimulating hormone highlights new genes, pathways and associations with thyroid disease


Alexander T Williams, Jing Chen, Kayesha Coley, Chiara Batini, Abril Izquierdo, Richard Packer, Erik Abner, Stavroula Kanoni, David J Shepherd, Robert C Free, Edward J Hollox, Nigel J Brunskill, Ioanna Ntalla, Nicola Reeve, Christopher E Brightling, Laura Venn, Emma Adams, Catherine Bee, Susan E Wallace, Manish Pareek, Anna L Hansell, Tõnu Esko, Estonian Biobank Research Team, Daniel Stow, Benjamin M Jacobs, David A van Heel, Genes & Health Research Team, William Hennah, Balasubramanya S Rao, Frank Dudbridge, Louise V Wain, Nick Shrine, Martin D Tobin, Catherine John



*Nat Commun*. 2023 Oct 23;14(1):6713.
doi: 10.1038/s41467-023-42284-5. PMID: 37872160


Brief summary: This study investigated genetic factors influencing normal variation in TSH levels (range 0.4 to 4.0 mIU/L) through a large genome-wide association study (GWAS) involving 247,107 European ancestry individuals. It identified 260 independent sentinel variants, of which 158 are novel. These variants explained 22.8% of TSH variance within the adult reference interval, implicating 112 putative causal genes, 76 of which had not been previously linked to TSH regulation. TSH polygenic scores were associated with thyroid diseases, including hypothyroidism, hyperthyroidism, and thyroid cancer. Testing biological pathways enrichment for the 112 putative causal genes highlighted classic signal transduction (G protein cAMP signaling), but also new pathways of interest like angiogenesis.

With the increasing ease of performing whole exome and genome sequencing, and the establishment of large biobanks, the number of published GWAS in thyroidology has rapidly increased. This study more than doubles the known genetic associations with TSH levels and highlights novel genes and pathways involved in thyroid regulation.

## 3.5. Multi-trait analysis characterizes the genetics of thyroid function and identifies causal associations with clinical implications

Rosalie B T M Sterenborg, Inga Steinbrenner, Yong Li, et al.

*Nat Commun*. 2024 Jan 30;15(1):888.

doi: 10.1038/s41467-024-44701-9. PMID: 38291025

Brief summary: This genome-wide association study (GWAS) meta-analysis of 271,040 European participants investigated the genetic determinants of thyroid function, including reference range serum TSH, FT4, free and total T3, proxies for metabolism (T3/FT4 ratio), as well as dichotomized high and low TSH levels. It identified 259 independent associations for TSH, 85 for FT4, and novel associations for thyroid hormone (TH) metabolism (T3/FT4 ratio). Genetic variants explained 14.1%, 6.0%, and 9.5% of TSH, FT4, and total T3 concentrations, respectively. Polygenic risk score and Mendelian randomization analyses showed that genetically determined variations in thyroid function have significant implications for cardiovascular diseases, autoimmune disorders, and cancer risk. Additionally, novel loci linked to TH transport, synthesis, and metabolism were identified.

In contrast to the study by Williams *et al* [1], this large-scale GWAS not only investigated genetic factors influencing TSH levels within the reference interval, but also reference range free FT4, FT3 and total T3, proxies for metabolism, and dichotomized high and low TSH levels. This provides new insights into the genetic regulation of thyroid function, as well as into TH transport, synthesis, and metabolism.

Reference

1. Alexander T Williams *et al*. Genome-wide association study of thyroid-stimulating hormone highlights new genes, pathways and associations with thyroid disease. *Nat Commun.* 2023 Oct 23;14(1):6713. doi: 10.1038/s41467-023-42284-5. PMID: 37872160

## 3.6. Genetic determinants of thyroid function in children

Tessa A Mulder, Purdey J Campbell, Peter N Taylor, Robin P Peeters, Scott G Wilson, Marco Medici, Colin Dayan, Vincent V W Jaddoe, John P Walsh, Nicholas G Martin, Henning Tiemeier, Tim I M Korevaar

*Eur J Endocrinol*. 2023 Aug 2;189(2):164-174.

doi: 10.1093/ejendo/lvad086. PMID: 37530217

Brief summary: This study investigated the genetic determinants of thyroid function in newborns and (pre)school children by analyzing the associations between single nucleotide polymorphisms (SNPs) previously identified in adults, and childhood TSH within the reference interval, and FT4 concentrations. It included three large population-based cohorts with data on genetic variants and thyroid function: Generation R (Netherlands), ALSPAC (UK), and BLTS (Australia), comprising 7,231 children. 30/60 “adult” TSH SNPs and 11/31 “adult” FT4 SNPs were also associated with thyroid function in childhood, with some SNPs (*AADAT*, *GLIS3*, *TM4SF4*, and *VEGFA*) exhibiting notably larger effect sizes in children compared to adults. Interestingly, genetic factors explained 5.3%-8.4% of TSH variability and 1.5%-4.2% of FT4 variability in children. Five TSH, but no FT4 SNPs were associated with thyroid function in neonates.

This study advances understanding of the genetic regulation of thyroid function in early life, showing that genetic variants associated with thyroid function in adults also influence childhood thyroid function, with some variants having stronger effects in children. However, more than 90% of TSH variability and more than 95% of FT4 variability in children cannot be explained by genetic factors. In a large Dutch twin study, Zwaveling-Soonawala *et al* found that environmental factors were the major factor influencing variability in neonatal

screening blood (total) T4 concentrations. Because T4 concentrations were measured on average on the fifth day of life, the fetal environment is the most likely candidate for the (shared) environmental influences on postnatal T4 concentrations, possibly by epigenetic modifications taking place in the fetal period [1].


Reference

1. Nitash Zwaveling-Soonawala 1, Catharina E M van Beijsterveldt 1, Ertirea T Mesfum 1, Brenda Wiedijk 1, Petra Oomen 1, Martijn J J Finken 1, Dorret I Boomsma 1, A S Paul van Trotsenburg 1. Fetal Environment Is a Major Determinant of the Neonatal Blood Thyroxine Level: Results of a Large Dutch Twin Study. *J Clin Endocrinol Metab.* 2015 Jun;100(6):2388-95. doi: 10.1210/jc.2015-1429. PMID: 25825950


## 3.7. The optimal healthy ranges of thyroid function defined by the risk of cardiovascular disease and mortality: systematic review and individual participant data meta-analysis


Yanning Xu, Arash Derakhshan, Ola Hysaj, et al for the Thyroid Studies Collaboration



*Lancet Diabetes Endocrinol*. 2023 Oct;11(10):743-754.

doi: 10.1016/S2213-8587(23)00227-9. PMID: 37696273


Brief summary: This large systematic review and individual participant data (IPD) meta-analysis investigated the association between serum TSH and FT4 concentrations and the risk of cardiovascular disease (CVD) events (coronary heart disease, stroke, and heart failure) and all-cause mortality. It included data from 134,346 participants, with a median age of 59 years (range 18-106) and a median follow-up of 11.5 years. The results indicate a J-shaped association between FT4 levels and CVD risk, with the lowest risk observed in the 20th to 40th percentiles of FT4 (13.5–14.8 pmol/L). Higher FT4 levels, particularly above the 85th percentile in women and the 75th percentile in men aged 70 and older, were associated with a more than 5% increase in the 10-year CVD risk. For TSH, the lowest risk was observed in the 60-80th percentiles (1.9–2.9 mIU/L), with both lower and higher TSH levels correlating with increased all-cause mortality.

In a comment in the same October issue of *Lancet Diabetes Endocrinol*, Elizabeth Pearce stated that "Xu and colleagues provide compelling evidence for working toward a more risk focused approach to defining normal thyroid function" [1]. Although the study by Xu *et al* certainly had strengths, it also had several limitations, and "adequately powered, long-term trials will be needed to establish age-specific and sex-specific thyroid function test reference ranges based on risk" [1]. An important question for pediatric endocrinologists is what these results mean for thyroid hormone treatment of children. In the treatment of children with severe primary congenital hypothyroidism, relatively high serum FT4 concentrations are often needed to normalize TSH [2].


References

1. Elizabeth N Pearce. Is it time to re-assess the development of thyroid function reference ranges? *Lancet Diabetes Endocrinol.* 2023 Oct;11(10):711-712. doi: 10.1016/S2213-8587(23)00257-7. Epub 2023 Sep 8. PMID: 37696274
2. van Trotsenburg P, Stoupa A, Léger J, Rohrer T, Peters C, Fugazzola L, Cassio A, Heinrichs C, Beauloye V, Pohlenz J, Rodien P, Coutant R, Szinnai G, Murray P, Bartés B, Luton D, Salerno M, de Sanctis L, Vigone M, Krude H, Persani L, Polak M. Congenital Hypothyroidism: A 2020-2021 Consensus Guidelines Update-An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid.* 2021 Mar;31(3):387-419. doi: 10.1089/thy.2020.0333. PMID: 33272083


# Congenital Hypothyroidism

## 3.8. Functional variants in a TTTG microsatellite on 15q26.1 cause familial nonautoimmune thyroid abnormalities


Satoshi Narumi, Keisuke Nagasaki, Mitsuo Kiriya, Erika Uehara, Kazuhisa Akiba, Kanako Tanase-Nakao, Kazuhiro Shimura, Kiyomi Abe, Chiho Sugisawa, Tomohiro Ishii, Kenichi Miyako, Yukihiro Hasegawa, Yoshihiro Maruo, Koji Muroya, Natsuko Watanabe, Eijun Nishihara, Yuka Ito, Takahiko Kogai, Kaori Kameyama, Kazuhiko Nakabayashi, Kenichiro Hata, Maki Fukami, Hirohito Shima, Atsuo Kikuchi, Jun Takayama, Gen Tamiya, Tomonobu Hasegawa



*Nat Genet*. 2024 May;56(5):869-876.

doi: 10.1038/s41588-024-01735-5. PMID: 38714868

Brief summary: The authors performed linkage analysis and whole-genome sequencing (WGS) of a Japanese family with both non-goitrous congenital hypothyroidism (CH) and multinodular goiter (MNG), and WGS in 10 other families. They discovered an association between these thyroid abnormalities and variants in a noncoding TTTG microsatellite at 15q26.1. Additional screening of 989 Japanese patients with CH showed that 13.9% carried a TTTG variant, that it was more prevalent in those with any family history of CH (41.5%), and that it was highly prevalent in a subgroup of CH patients with parent-to-offspring transmission of CH (75.0%).

The clinical phenotype of variant-carrying patients was relatively uniform: moderately elevated serum TSH levels in combination with FT4 usually within the reference interval, but elevated thyroglobulin levels in almost all, and slightly small thyroid glands in most patients. Functional assays suggested that the TTTG microsatellite is a thyroid-specific repressor and that sequence changes affecting the microsatellite cause loss of repressor activity. The precise mechanism causing the non-goitrous CH and the MNG, remains to be elucidated.

## 3.9. STR mutations on chromosome 15q cause thyrotropin resistance by activating a primate-specific enhancer of MIR7-2/MIR1179

Helmut Grasberger, Alexandra M Dumitrescu, Xiao-Hui Liao, Elliott G Swanson, Roy E Weiss, Panudda Srichomkwun, Theodora Pappa, Junfeng Chen, Takashi Yoshimura, Phillip Hoffmann, Monica Malheiros França, Rebecca Tagett, Kazumichi Onigata, Sabine Costagliola, Jane Ranchalis, Mitchell R Vollger, Andrew B Stergachis, Jessica X Chong, Michael J Bamshad, Guillaume Smits, Gilbert Vassart, Samuel Refetoff

*Nat Genet*. 2024 May;56(5):877-888.
doi: 10.1038/s41588-024-01717-7. PMID: 38714869

Brief summary: This study investigated congenital hypothyroidism (CH) due to dominantly inherited resistance to TSH (RTSH) in 12 unrelated families. It reveals that mutations in a non-coding (TTTG) short tandem repeat (STR) on chromosome 15q cause this condition by activating a thyroid-specific enhancer cluster. Functional studies showed that activation of this enhancer cluster leads to upregulation of the bicistronic MIR7-2/MIR1179 locus, resulting in overexpression of its microRNAs in thyroid cells. This dysregulation probably alters thyroid signaling pathways, with both anti-proliferative and proliferative effects.

The study included genetic analysis of 148 individuals of whom 68 carried mutations. Just like the study by Narumi *et al* [1], the clinical phenotype of mutation carriers consisted of elevated serum TSH and thyroglobulin levels, in combination with FT4 levels within the reference interval. Also like Narumi *et al*, several mutation carriers developed enlarged nodular thyroid glands, with three participants requiring thyroid surgery [1].

The studies by Narumi *et al* and Grasberger *et al*, both published in the May 2024 issue of *Nature Genetics*, are the first to report the same novel genetic cause of CH caused by pathogenic variants in *non-coding* DNA. Narumi *et al*. suggest that it is a frequent cause of CH with a gland-in-situ, especially when the family history is positive for CH or multinodular goiter. Over the last several years, at least five Mendelian disorders have been linked to mutations in miRNA stem loops [2-5], or deletion of a miRNA cluster [6], but STR mutation-linked RTSH appears to be uniquely caused by abnormal *pri-MIR* expression. Given that 98-99% of the human genome consist of non-coding DNA, it is likely that many more conditions - including CH - will be explained in this way in the coming years.

References

1. Satoshi Narumi *et al*. Functional variants in a TTTG microsatellite on 15q26.1 cause familial nonautoimmune thyroid abnormalities. *Nat Genet.* 2024 May;56(5):869-876. doi: 10.1038/s41588-024-01735-5. Epub 2024 May 7. PMID: 38714868
2. Mencía A, Modamio-Høybjør S, Redshaw N, Morín M, Mayo-Merino F, Olavarrieta L, Aguirre LA, del Castillo I, Steel KP, Dalmay T, Moreno F, Moreno-Pelayo MA. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. *Nat Genet.* 2009 May;41(5):609-13. doi: 10.1038/ng.355. Epub 2009 Apr 12. PMID: 19363479
3. Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, Simpson DA, Willoughby CE. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. *Am J Hum Genet.* 2011 Nov 11;89(5):628-33. doi: 10.1016/j.ajhg.2011.09.014. Epub 2011 Oct 11. PMID: 21996275
4. MiR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma Affiliations Expand. Ivan Conte, Kristen D Hadfield, Sara Barbato, Sabrina Carrella, Mariateresa Pizzo, Rajeshwari S Bhat, Annamaria Carissimo, Marianthi Karali, Louise F Porter, Jill Urquhart, Sofie Hateley, James O'Sullivan, Forbes D C Manson, Stephan C F Neuhauss, Sandro Banfi, Graeme C M Black. *Proc Natl Acad Sci U S A*. 2015 Jun 23;112(25):E3236-45. doi: 10.1073/pnas.1401464112. Epub 2015 Jun 8. PMID: 26056285

5. Giedre Grigelioniene, Hiroshi I Suzuki, Fulya Taylan, Fatemeh Mirzamohammadi, Zvi U Borochowitz, Ugur M Ayturk, Shay Tzur, Eva Horemuzova, Anna Lindstrand, Mary Ann Weis, Gintautas Grigelionis, Anna Hammarsjö, Elin Marsk, Ann Nordgren, Magnus Nordenskjöld, David R Eyre, Matthew L Warman, Gen Nishimura, Phillip A Sharp, Tatsuya Kobayashi. Gain-of-function mutation of microRNA-140 in human skeletal dysplasia. *Nat Med.* 2019 Apr;25(4):583-590. doi: 10.1038/s41591-019-0353-2. Epub 2019 Feb 25. PMID: 30804514
6. Loïc de Pontual, Evelyn Yao, Patrick Callier, Laurence Faivre, Valérie Drouin, Sandra Cariou, Arie Van Haeringen, David Geneviève, Alice Goldenberg, Myriam Oufadem, Sylvie Manouvrier, Arnold Munnich, Joana Alves Vidigal, Michel Vekemans, Stanislas Lyonnet, Alexandra Henrion-Caude, Andrea Ventura, Jeanne Amiel. Germline deletion of the miR-17∼92 cluster causes skeletal and growth defects in humans. *Nat Genet.* 2011 Sep 4;43(10):1026-30. doi: 10.1038/ng.915. PMID: 21892160

## Defects in Thyroid Hormone Transport, Metabolism and Action

### 3.10. Resistance to thyroid hormone induced tachycardia in RTHα syndrome

Riccardo Dore, Laura Watson, Stefanie Hollidge, Christin Krause, Sarah Christine Sentis, Rebecca Oelkrug, Cathleen Geißler, Kornelia Johann, Mehdi Pedaran, Greta Lyons, Nuria Lopez-Alcantara, Julia Resch, Friedhelm Sayk, Karl Alexander Iwen, Andre Franke, Teide Jens Boysen, Jeffrey W Dalley, Kristina Lorenz, Carla Moran, Kirsten L Rennie, Anders Arner, Henriette Kirchner, Krishna Chatterjee, Jens Mittag

*Nat Commun*. 2023 Jun 7;14(1):3312.
doi: 10.1038/s41467-023-38960-1. PMID: 37286550

Brief summary: This study examined resistance to thyroid hormone (TH)-induced tachycardia in patients with Resistance to THα (RTHα). This rare condition is caused by mutations in the TH receptor alpha (TRα1), and leads to hypothyroidism in TRα1-expressing tissues, including the heart. While high doses of thyroxine (T4) are typically used to treat this condition, patients with RTHα do not exhibit the expected increase in heart rate despite overcoming tissue resistance to the hormone.

The authors used telemetry in a mouse model harboring the TRα1 mutation (TRα1+m), and found that the persistent bradycardia is due to an intrinsic cardiac defect rather than altered autonomic control. Although TH typically upregulates pacemaker channels (Hcn2 and Hcn4) involved in heart rate regulation, the study demonstrated that these channels are upregulated normally in RTHα patients and TRα1+m mice, but that the expression of other key ion channel genes, including potassium channel genes such as *Kcnh2* as well as the calcium channel *Ryr2*, was irreversibly altered. Further investigation showed that fetal exposure to high levels of triiodothyronine (T3) via the mothers of TRα1+m animals restored altered expression and DNA methylation of ion channels, including Ryr2.

These findings suggest that the resistance to TH-induced tachycardia in RTHα syndrome results from altered expression of several key ion channel genes, probably caused by altered DNA methylation. These observations provide a mechanistic understanding for the lack of tachycardia in thyroxine-treated RTHα patients, and suggest that high dose hormone therapy of this disorder may be safer than previously thought.

### 3.11. Identification of human TRIAC transmembrane transporters

Paul Carlos Becker, Mandy Güth-Steffens, Katina Lazarow, Niklas Sonntag, Doreen Braun, Islam Masfaka, Kostja Renko, Lutz Schomburg, Josef Köhrle, Jens Peter von Kries, Ulrich Schweizer, Gerd Krause, Jonas Protze

*Thyroid*. 2024 Jul;34(7):920-930.
doi: 10.1089/thy.2023.0592. PMID: 38801167

Brief summary: This basic science study examined how 3,5,3'-triiodothyroacetic acid (TRIAC) - a thyroid hormone (TH) receptor agonist used in patients with resistance to TH, and in patients with Allan-Herndon-Dudley syndrome (AHDS), caused by mutations in monocarboxylate transporter 8 (MCT8) - is transported into cells independent of MCT8. Through a genome-wide RNAi screening in HepG2 cells, two key transporters, SLC22A9 (OAT7) and SLC29A2 (ENT2), were identified that facilitate the uptake of TRIAC into cells. These transporters are expressed in tissues like brain, liver, and pituitary, suggesting their role in enabling TRIAC to cross cellular membranes and exert its effects in these tissues. Furthermore, ABCD1, an ATP-dependent peroxisomal pump, was identified as a TRIAC exporter, which may limit the efficacy of TRIAC therapy in certain tissues.

These findings have clear clinical relevance, particularly in conditions such as AHDS, where TRIAC treatment has variable results. Better understanding of the expression patterns and function of these transporters may improve the therapeutic use of TRIAC in pediatric patients with TH transport disorders. The study also highlights the importance of screening for transporter expression in other tissues to optimize treatment outcomes.

## Thyroid Autoimmunity

### 3.12. The genetics of Graves' disease

Lydia Grixti, Laura C Lane, Simon H Pearce
*Rev Endocr Metab Disord*. 2024 Feb;25(1):203-214.
doi: 10.1007/s11154-023-09848-8. PMID: 38108994

Brief summary: This article provides an in-depth review of the genetic factors contributing to Graves' disease (GD), the most common cause of hyperthyroidism. The authors explain that GD has a strong genetic component, with genetic factors accounting for 60-80% of the risk of developing this disease. The review traces the progress of genetic studies from initial candidate gene studies to genome-wide association studies that have identified over 80 susceptibility loci. Key genetic variants, such as those in *HLA*, *CTLA4*, and *PTPN22*, have been found to play significant roles in GD susceptibility. The authors also discuss emerging genotype-phenotype correlations, including how genetic variants might influence clinical outcomes such as disease severity, age of onset, and relapse rates. These findings could eventually contribute to personalized approaches in GD treatment.

While GD is largely influenced by genetic factors, the identification of numerous susceptibility loci indicates a complex, polygenic inheritance pattern. Further large-scale studies are required to validate these findings and explore their clinical implications in precision medicine for GD.

### 3.13. Epigenome-wide association study shows differential DNA methylation of *MDC1*, *KLF9*, and *CUTA* in autoimmune thyroid disease

Nicole Lafontaine, Christopher J Shore, Purdey J Campbell, Benjamin H Mullin, Suzanne J Brown, Vijay Panicker, Frank Dudbridge, Thomas H Brix, Laszlo Hegedüs, Scott G Wilson, Jordana T Bell, John P Walsh
*J Clin Endocrinol Metab*. 2024 Mar 15;109(4):992-999.
doi: 10.1210/clinem/dgad659. PMID: 37962983

Brief summary: This epigenome-wide association study (EWAS) aimed to identify differentially methylated positions (DMPs) and regions (DMRs) between Graves' disease (GD) and Hashimoto's disease (HD) in two independent patient cohorts from Australia (30 patients with GD and 30 with HD, discovery cohort) and Denmark (32 patients with GD and 32 with HD, replication cohort). Linear mixed models were used to test for differences in quantile-normalized β values of DNAm between GD and HD and data were later meta-analyzed. The study identified two DMPs with significant differences in methylation: one within the *KLF9* gene, which is associated with thyroid hormone regulation, and another in *MDC1*, a gene involved in immune responses. Additionally, a differentially methylated region within the *CUTA* gene was replicated in both cohorts.

These findings suggest that epigenetic modifications, particularly DNAm, play a role in the development and clinical presentation of AITD, potentially influencing whether a patient develops GD or HD. *KLF9* has been previously associated with thyroid hormone levels, while *MDC1* is located in a region of the genome known to be important for immune regulation, particularly in T- and B-cell function.

These epigenetic differences could potentially serve as biomarkers for distinguishing between GD and HD, and may contribute to better understanding of how environmental factors interact with genetic predisposition to influence disease expression.

## Thyroid Cancer

### 3.14. Polygenic risk scores, radiation treatment exposures and subsequent cancer risk in childhood cancer survivors

Todd M Gibson, Danielle M Karyadi, Stephen W Hartley, Michael A Arnold, Amy Berrington de Gonzalez, Miriam R Conces, Rebecca M Howell, Vidushi Kapoor, Wendy M Leisenring, Joseph P Neglia, Joshua N Sampson, Lucie M Turcotte, Stephen J Chanock, Gregory T Armstrong, Lindsay M Morton

*Nat Med*. 2024 Mar;30(3):690-698.
doi: 10.1038/s41591-024-02837-7. PMID: 38454124

Brief summary: This study explored the combined effect of genetic predisposition and radiation exposure on the risk of developing subsequent cancers in survivors of childhood cancer. It included genotype data from 11,220 5-year survivors and focused on six subsequent cancer types: basal cell carcinoma (BCC), breast cancer, thyroid cancer, squamous cell carcinoma (SCC), melanoma, and colorectal cancer. Polygenic risk scores (PRS) were derived from genome-wide association studies (GWAS) in the general population and were applied to the survivors to assess their predictive value in this unique population. PRS were associated with the risk of subsequent cancers, particularly BCC (odds ratio per s.d. of the PRS: OR =1.37, 95% confidence interval (CI) =1.29–1.46), breast cancer (OR =1.42, 95% CI =1.27–1.58), thyroid cancer (OR =1.48, 95% CI =1.31–1.67), and melanoma (OR =1.60, 95% CI =1.31–1.96).

An important finding is that the joint effects of radiation exposure and PRS are more than additive. therefore, survivors with both high radiation exposure and high PRS have a particularly elevated risk of developing these cancers. For example, the cumulative incidence of subsequent thyroid cancer by age 50 was 18% for those exposed to high neck radiation (10-30 Gy) and with a high PRS, compared to 6% for those with similar radiation exposure but a low PRS.

Integrating genetics with treatment history may provide valuable insights for risk stratification in childhood cancer survivors, particularly those at higher risk due to radiation exposure. These findings could influence long-term follow-up guidelines and cancer surveillance strategies in this population.

Currently, the recommendation for thyroid cancer surveillance in survivors of childhood, adolescent, and young adult (CAYA) cancer are counseling regarding risk and surveillance options for differentiated thyroid carcinoma, at least every 5 years. If the decision to commence surveillance is made, a shared decision should be made for one of these two surveillance modalities:

- neck palpation, every 1 to 2 years, starting 5 years after radiotherapy, or
- thyroid ultrasonography, every 3 to 5 years, starting 5 years after radiotherapy [1].

If genotyping and PRS calculation become regular care, an interesting question is whether and how this will change current thyroid cancer surveillance in CAYA cancer survivors: more intensive in those with a higher risk, or less intensive in those with a lower risk?

Reference

1. Laura van Iersel, Renee L Mulder, Christian Denzer, *et al*. Hypothalamic-Pituitary and Other Endocrine Surveillance Among Childhood Cancer Survivors. *Endocr Rev*. 2022 Sep 26;43(5):794-823. doi: 10.1210/endrev/bnab040. PMID: 34962573

## Thyroid Tissue Regeneration

### 3.15. Progress toward and challenges remaining for thyroid tissue regeneration

Mírian Romitti, Sabine Costagliola

*Endocrinology*. 2023 Aug 28;164(10):bqad136.
doi: 10.1210/endocr/bqad136. PMID: 37690118

Brief summary: The article provides a comprehensive overview of current advancements and remaining obstacles in thyroid tissue regeneration.

Recent studies have identified potential cellular mechanisms that may contribute to thyroid regeneration following partial tissue damage, including thyroid progenitor cells and microfollicles. Yet, regeneration after damage remains limited as the gland has a slow cell turnover rate. Moreover, in cases of total thyroidectomy or congenital absence (thyroid agenesis), regeneration does not occur, highlighting the need for external sources of thyroid tissue. Thyroid organoid transplantation has emerged as a promising approach for restoring thyroid function. Researchers have successfully generated thyroid organoids from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult thyroid tissue, showing functionality in preclinical models.

This research holds potential for future clinical applications, particularly in pediatric patients with Thyroid Tissue Regeneration or those requiring thyroid replacement therapy following surgery. While significant progress has been made, challenges remain in achieving full maturation and functionality of human thyroid organoids in vitro and in vivo. Other points of attention are long-term safety, efficacy, and the immune response to transplanted organoids.

## Thyroid Imaging

### 3.16. A fully autonomous robotic ultrasound system for thyroid scanning

Kang Su, Jingwei Liu, Xiaoqi Ren, Yingxiang Huo, Guanglong Du, Wei Zhao, Xueqian Wang, Bin Liang, Di Li, Peter Xiaoping Liu
*Nat Commun*. 2024 May 11;15(1):4004.
doi: 10.1038/s41467-024-48421-y. PMID: 38734697

Brief summary: These authors developed a fully autonomous robotic ultrasound (US) system for thyroid scanning (FARUS). The system consists of a six-degree-of-freedom UR3 manipulator that carries a linear US probe, a US probe fixture, a six-axis force/torque sensor and a Kinect camera. Using reinforcement learning, Bayesian optimization, and deep learning, the system dynamically adjusts the probe position and performs real-time segmentation of thyroid tissue and potential nodules. 500 contrast-enhanced head and neck CT images were used for the estimation of the thyroid scanning range, and FARUS was trained and optimized by comparing manually collected thyroid ultrasonography data to ultrasonography data obtained by FARUS in 165 healthy volunteers (from college students to elderly individuals). A clinical study in 19 adult patients (age $53.05 \pm 5.90$ years) shows that FARUS can achieve results comparable to manual scans performed by experienced clinicians. FARUS can also assess thyroid nodules based on the American College of Radiology (ACR) Thyroid Imaging Reporting and Data System (TI-RADS) guidelines, assisting in the diagnosis of malignant nodules. This new technology offers a non-invasive, consistent, and reliable solution for thyroid screening.

This is the first in-human study of fully autonomous robotic US scanning of the thyroid gland. In 2022, Zhang *et al* reported results of a feasibility study of a 5G-based telerobotic US system for thyroid examination on a rural Island, with the patient's subsystem and the subsystem operated by a tele-doctor at another hospital connected by the fast and large-bandwidth 5G network [1]. However, the autonomous nature of FARUS minimizes operator dependency and, with that, may be especially valuable for rapid outpatient screening or in resource-limited settings.

Reference

1. Ya-Qin Zhang, Hao-Hao Yin, Tian He, Le-Hang Guo, Chong-Ke Zhao, Hui-Xiong Xu. Clinical application of a 5G-based telerobotic ultrasound system for thyroid examination on a rural island: a prospective study. *Endocrine.* 2022 Jun;76(3):620-634. doi: 10.1007/s12020-022-03011-0. PMID: 35182363

# 4. Growth and Growth Factors

Valentina Pampanini[1,2], Annalisa Deodati[1,3], Maria Elisa Amodeo[1], Elena Inzaghi[1], Patricia-Maria Anton[4], Stefano Cianfarani[1,2,3]

[1] IRCCS "Bambino Gesù" Children's Hospital, Rome, Italy. [2] Department of Systems Medicine, University of Rome 'Tor Vergata', Rome, Italy; [3] Department of Women's and Children's Health, Karolinska Institute and University Hospital, Stockholm, Sweden; [4]National Institute of Endocrinology "CI Parhon", Bucharest, Romania

Address correspondence to: Stefano Cianfarani IRCCS "Bambino Gesù" Children's Hospital P.zza S. Onofrio, 4 00165, Roma, Italy E-mail: stefano.cianfarani@uniroma2.it Tel. +39 06 6859 3074 (Hosp.)/+39 06 72596178 (Lab.) Fax. +39 06 6859 2508 (Hosp.)/+39 06 72596172 (Lab.)

## Important for Clinical Practice

### 4.1. Accuracy of glucagon testing across transition in young adults with childhood-onset growth hormone deficiency

Fava D, Guglielmi D, Pepino C, Angelelli A, Casalini E, Varotto C, Panciroli M, Tedesco C, Camia T, Naim A, Allegri AEM, Patti G, Napoli F, Gastaldi R, Parodi S, Salerno MC, Maghnie M, Di Iorgi N
*J Clin Endocrinol Metab*. 2024 Jun 24:dgae408.
doi: 10.1210/clinem/dgae408. PMID: 38913686

Brief Summary: This study identifies a reliable cut-off value for the glucagon stimulation test, which may be used as an alternative to the insulin tolerance test (ITT) in the diagnosis of growth hormone deficiency (GHD) in transition age.

Many patients with childhood-onset growth hormone deficiency (GHD) show normal GH secretion when re-tested at the end of growth, especially those with isolated GHD and normal or small pituitary gland (1). ITT is recognized as the gold standard test for the diagnosis of GHD in transition age, with a cut-off value $\leq$ 6 μg/L (2). However, ITT should be avoided in patients with epilepsy, cerebral or cardiovascular diseases (3); in such cases, the glucagon stimulation test (GST) is a valid alternative to ITT. For the diagnosis of adult GHD, the American Association of Clinical Endocrinologists (AACE) recommend using a GH cut-off of 3 μg/L for normal and overweight patients with a high pretest probability of GHD or 1 μg/L for overweight and obese patients with a low pretest probability of GHD (4). However, these cut-off values are not been established in clinical practice for the transition phase.

This study evaluated the accuracy of the glucagon stimulation test (GST) compared to ITT in the diagnosis of GHD in young adults with childhood-onset GHD in 97 patients (median age, 17.39 years) who underwent ITT, GST, and IGF-1 testing. Patients were affected by a) idiopathic isolated GHD ($n=44$); b) moderate organic GHD, with congenital anomalies (pituitary stalk interruption syndrome and other cerebral midline defects), organic hypothalamic-pituitary disease (secondary to brain surgery, craniospinal and total body irradiation, or infiltrative disease of the hypothalamus and pituitary stalk), with 1 or 2 pituitary defects ($n=35$) and severe organic GHD ($\geq 3$ hormone deficiencies, $n=18$).

ROC curve analysis of GST showed a GH peak value of 7.3 μg/L as the optimal cutoff (95% CI 4.15-8.91; sensitivity 95.7%, specificity 88.2%) to correctly classify 91.8% of the entire cohort. Subgroup analyses were possible only in moderate organic GHD patients (highly likelihood GHD), due to the insufficient sample size in the severe organic GHD group. The proportion of correctly classified patients at the optimal threshold (91.4%) was obtained for a GH peak cutoff of 5.8 μg/L (95% CI 3.16-7.39; sensitivity, 96.0%, specificity 80.0%). A strong concordance between GST and ITT was reported, as demonstrated by an intraclass correlation coefficient around 92%.

GST is a safe alternative to ITT for assessing GH secretion in young adults with childhood-onset GHD. Patients with a GH peak $<$ 5.8 μg/L are candidates to restart rhGH therapy.

References

1. Laurer E, Sirovina A, Blaschitz A, *et al*. The landscape of retesting in childhood-onset idiopathic growth hormone deficiency and its reversibility: a systematic review and meta-analysis. *Eur J Endocrinol.* 2022;187(2):265-278.
2. Ho KKY; 2007 GH Deficiency Consensus Workshop Participants. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *Eur J Endocrinol*. 2007;157(6):695-700.
3. Yeoh P, Dwyer AA, Anghel E, *et al*. A comparison of the blood glucose, growth hormone, and cortisol responses to two doses of insulin (0.15 U/kg vs. 0.10 U/kg) in the insulin tolerance test: a single-centre audit of 174 cases. *Int J Endocrinol.* 2022;2022: 7360282.
4. Yuen KCJ, Biller BMK, Radovick S, *et al*. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of growth hormone deficiency in adults and patients transitioning from pediatric to adult care. *Endocr Pract.* 2019;25(11):1191-1232.

## 4.2. The impact of prenatal alcohol exposure on longitudinal growth, nutritional status, and insulin-like growth factor 1 in early childhood in Leyte, the Philippines

Barry CV, Chrysanthopoulou SA, Tallo V, Jarilla B, Vargas Z, McDonald E, Gundogan F, Friedman JF

*J Pediatr.* 2024 Jun;269:113977.

doi: 10.1016/j.jpeds.2024.113977. PMID: 38401788

Brief Summary: This longitudinal study describes the long-term effects of prenatal alcohol exposure on early childhood development. The results clearly show that prenatal alcohol exposure impairs growth and IGF-1 levels in children, highlighting the need for public health interventions to prevent alcohol consumption during pregnancy, especially in low-resource settings.

This study assessed the long-term consequences of prenatal alcohol exposure (PAE) on early childhood development in Leyte, Philippines. It provides insights into strategies for the timely identification of cases and potential mechanistic pathways of an often underrepresented issue in global health research. (1). It followed a cohort of 296 mother-infant couples (32% cases and 68% controls) from early gestation through 24 months of age, measuring child's auxological parameters, IGF-I, leptin and serum phosphatidylethanol (PEth), a direct biomarker of alcohol metabolism of mothers and infants (2).

Children exposed to alcohol in utero had impaired growth trajectories compared to their non-exposed peers, in particular in height-for-age z-score. The effect appeared between 4 and 6 months of age and continued through 12-24 months, together with a decreased rate of mid-upper-arm circumference growth from birth to 12 months. This finding is consistent with previous studies but adds depth by linking these growth impairments to both nutritional status and levels of IGF-I that were significantly lower at birth and 6 months in children with PAE (3-4). The association between PAE and lower IGF-I levels suggests a possible mechanism through which PAE may lead to stunting (5).

These findings could drive future interventions aimed at mitigating the effects of PAE, such as nutritional supplementation or targeted therapies to boost IGF-1 levels in affected children. Furthermore, this study draws attention to the broader public health implications of alcohol abuse during pregnancy, particularly in settings where malnutrition and poor health infrastructure pose significant threats to child development.

References

1. Burd L. Fetal alcohol spectrum disorder: complexity from comorbidity. *Lancet.* 2016 Mar 5;387(10022):926-927. doi: 10.1016/S0140-6736(15)01346-X. Epub 2016 Jan 6. PMID: 26777271.
2. Bakhireva LN, Leeman L, Savich RD, Cano S, Gutierrez H, Savage DD, Rayburn WF. The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure. *Alcohol Clin Exp Res.* 2014 Apr;38(4):1078-85. doi: 10.1111/acer.12349. Epub 2014 Feb 11. PMID: 24511895; PMCID: PMC4412471.
3. Savage MO, Burren CP, Rosenfeld RG. The continuum of growth hormone-IGF-I axis defects causing short stature: diagnostic and therapeutic challenges. *Clin Endocrinol (Oxf).* 2010 Jun;72(6):721-8. doi: 10.1111/j.1365-2265.2009.03775.x. Epub 2009 Dec 29. PMID: 20050859.
4. Ni Q, Tan Y, Zhang X, Luo H, Deng Y, Magdalou J, Chen L, Wang H. Prenatal ethanol exposure increases osteoarthritis susceptibility in female rat offspring by programming a low-functioning IGF-1 signaling pathway. *Sci Rep.* 2015 Oct 5;5:14711. doi: 10.1038/srep14711. PMID: 26434683; PMCID: PMC4592973.
5. Han VK, Lund PK, Lee DC, D'Ercole AJ. Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. *J Clin Endocrinol Metab.* 1988 Feb;66(2):422-9. doi: 10.1210/jcem-66-2-422. PMID: 2448331.

## 4.3. A clinical trial of high dose growth hormone in a patient with a dominant negative growth hormone receptor mutation

Merchant N, Houchin L, Boucher K, Dauber A
*J Clin Endocrinol Metab*. 2024 Apr 10:dgae244.
doi: 10.1210/clinem/dgae244. PMID: 38597155

Brief Summary: This single-patient intervention study showed the efficacy of high-dose rhGH treatment in overcoming GH resistance in a child harboring a dominant-negative GH receptor (GHR) mutation.

GH insensitivity includes a broad spectrum of defects (1). Laron syndrome is the best-known GH insensitivity syndrome, characterized by recessive mutations in GHR and good response to IGF-I treatment (2). Dominant-negative variants of GHR are extremely rare and affect different domains of the receptor. Usually, the extracellular binding domain of GHR can be cleaved into GH binding protein (GHBP) which acts as carrier protein for circulating GH. Mutations affecting the extracellular domain may thus influence GHBP levels as well as GH signaling.

In this case report, rhGH was administered to a boy carrying a heterozygous variant of GHR, located in the extracellular domain. The patient had high GHBP levels, consistent with higher degree of truncated protein caused by the mutation. The patient had severe short stature (-3.18 SDS at the age of 9 years and 9 months) and biochemical features consistent with GH resistance (high GH peak under stimulation test and reduced IGF-I levels). The patient's father, who had the same mutation and severe short stature, had been unsuccessfully treated with GH at normal dosage. In this trial high rhGH doses were used to overcome GH resistance. To achieve an IGF-1 serum concentration above the mean but below +2 SDS, a dose escalation phase was employed, whereby the GH dosage was progressively increased from 50 µg/kg/day to the maximum dose of 250 µg/kg/day over a period of 2.5 months. This was followed by a dose-stable phase, resulting in a total treatment period of 12 months. Height velocity increased from 5.3 cm/year to 8.7 cm/year, leading to a height gain of 0.81 SDS (-3.18 to -2.37 SDS). Notably, no significant advancement of bone age or adverse effects were reported. An extension of the therapeutic phase is still ongoing and will provide further data on long-term safety and efficacy.

References

1. Mastromauro C, Giannini C, Chiarelli F. Short stature related to Growth Hormone Insensitivity (GHI) in childhood. *Front Endocrinol (Lausanne).* 2023 15:14:1141039.
2. Brooks AJ, Waters MJ. The growth hormone receptor: mechanism of activation and clinical implications. *Nat Rev Endocrinol.* 2010;6(9):515-25.

## 4.4. Recombinant human insulin-like growth factor-1 treatment of severe growth failure in three siblings with STAT5B deficiency

Muthuvel G, Al Remeithi SS, Foley C, Dauber A, Hwa V, Backeljauw P
*Horm Res Paediatr.* 2024;97(2):195-202.
doi: 10.1159/000531491. PMID: 37586336

Brief Summary: This case series describes the effect of recombinant human IGF-1 (rhIGF-1) administration on growth of three siblings with STAT5B homozygous recessive mutations.

The peripheral effects of GH are primarily mediated by IGF-I through the activation of the GH receptor (GHR)-signal transducer and activator of transcription (STAT)-5B signaling. Patients carrying *STAT5B* mutations have severe postnatal growth failure and IGF-I deficiency associated with immunodeficiency and increased risk of autoimmune and pulmonary disease (1). GH resistance makes rhGH treatment ineffective in stimulating growth (2).

The three siblings harboring a homozygous inactivating mutation in *STA5B* were evaluated for severe short stature (height at baseline: -6.5; -4.9, -5.3 SDS). All had reduced IGF-I (< -2.5 SDS) and IGFBP-3 (< -3 SDS) levels and received rhIGF-1 treatment under FDA guidelines for severe primary IGF-I deficiency, at an early age (4.9, 2.8, and 2.1 years). The duration of follow-up was up to 6 years. The starting dose was 40 µg/kg/dose

twice daily subcutaneously and progressively increased up to 110-120 μg/kg/dose twice daily and then was modulated according to side effects. Height velocity (cm/year) and height gain (SDS) were the main outcome measures. Patient 1 had baseline height velocity of 3 cm/year, thereafter 5.7 cm/year, 6 cm/year, 5.2 cm/year and 4.7 cm/year in the first, second, third and sixth year of treatment, respectively. The overall height gain was +2.21 SDS after 6 years of treatment (height from -6.87 to -4,66 SDS). Patient 2 had pretreatment height velocity of 3 cm/year, and 7.1 cm/year, 4.9 cm/year, 4.8 cm/year and 3.8 cm/year in the first, second, third and sixth year of treatment, respectively. The overall height gain was +0.93 SDS SDS after 6 years of treatment (height from -5.87 to -4.94 SDS). Patient 3 had pretreatment height velocity of 5.2 cm/year, and 7.4 cm/year, 5.5 cm/year and 4.3 cm/year in the first, second and fifth year of treatment, respectively. The overall height change was – 0.62 SDS after 5 years of treatment (height from -5.86 to -6.48 SDS). No major side effects were observed during treatment in all patients.

Available data on the efficacy of rhIGF-I for increasing growth in patients with *STAT5B* inactivating mutations are scant and inconclusive (3). These results show that rhIGF-I therapy may be effective in stimulating growth and partial catch-up growth, especially in the first year of treatment and may represent a therapeutic option to avoid worsening of growth failure in subjects with inactivating *STAT5B* mutations.

References


1. Hwa V. STAT5B deficiency: Impacts on human growth and immunity. *Growth Horm IGF Res.* 2016:28:16-20.
2. Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, Pratt KL, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ, Rosenfeld RG. Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med.* 2003 18;349(12):1139-47.
3. Klammt J, Neumann D, Gevers EF, Andrew SF, Schwartz D, Rockstroh D, Colombo R, Sanchez MA, Vokurkova D, Kowalczyk J, Metherell L, Rosenfeld R, Pfäffle R, Dattani M, Dauber A, Hwa V. Dominant-negative STAT5B mutations cause growth hormone insensitivity with short stature and mild immune dysregulation. *Nat Commun.* 2018 29;9(1):2105.


## 4.5. Cerebrovascular abnormalities in adults born SGA at 12 years after growth hormone cessation compared to controls


Dorrepaal DJ, Goedegebuure WJ, Smagge L, van der Steen M, van der Lugt A, Hokken-Koelega ACS



*J Clin Endocrinol Metab*. 2024 Feb 20;109(3):e1185-e1193.
doi: 10.1210/clinem/dgad622. PMID: 37855389


Brief Summary: This single-center, prospective study used brain MRI to assess the prevalence of cerebrovascular abnormalities in a large cohort of adult patients born SGA and treated with GH during childhood, 12 years after rhGH treatment cessation. GH treatment was not associated with a higher incidence of aneurysms, intracerebral hemorrhages, microbleeds or other vascular abnormalities.

The French population-based cohort of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study reported increased cerebrovascular morbidity due to hemorrhagic stroke in GH-treated subjects, including those born SGA, compared with the general population (1). The main limitation of SAGhE was the absence of an appropriate control group of untreated SGA subjects to distinguish whether the increased cerebrovascular morbidity was linked to GH treatment or secondary to the underlying condition leading to be born SGA.

The current study investigated cerebrovascular abnormalities using MRI in SGA adults treated with rhGH (SGA-GH) 12 years after GH cessation (mean GH treatment duration 8.3±2.3 years), around the age of 30 years, in comparison with 3 untreated age-matched control groups: untreated short adults born SGA (SGA-S), adults born SGA with spontaneous catch-up growth and normal height (SGA-CU), and AGA adults.

The study population consisted of 301 patients, of whom 94 SGA-GH, 42 SGA-S, 69 SGA-CU and 96 AGA. Aneurysms were found in 6 adults: 3 (3.6%) SGA-GH, 1 (2.9%) SGA-S and 2 (2.2%) AGA adults, without significant differences between SGA-GH and controls. Previous intracerebral hemorrhages were found in 2 SGA-S adults (4.8%). Microbleeds were found in 17 adults: 4 (4.3%) SGA-GH, 4 (9.5%) SGA-S, 3 (4.3%) SGA-CU and 6 (6.3%) AGA adults, without significant differences between SGA-GH and controls.

In conclusion, this prospective case-control study shows that rhGH treated SGA patients do not have an increased risk of cerebrovascular abnormalities 12 years after GH therapy in comparison with closely matched controls.

Reference

1. Poidvin A, Touzé E, Ecosse E, Landier F, Béjot Y, Giroud M, Rothwell PM, Carel JC, Coste J. Growth hormone treatment for childhood short stature and risk of stroke in early adulthood. *Neurology*. 2014 Aug 26;83(9):780-6. doi: 10.1212/WNL.0000000000000737. Epub 2014 Aug 13. PMID: 25122206.

## 4.6. Clinical characteristics of pathogenic *ACAN* variants and 3-year response to growth hormone treatment: real-world data

Renes JS, Reedijk AMJ, Losekoot M, Kant SG, Van der Steen M, Van der Kaay DCM, Hokken-Koelega ACS, Van Duyvenvoorde HA, de Bruin C

*Horm Res Paediatr.* 2024 Jan 17:1-14.
doi: 10.1159/000535651. PMID: 38232712

Brief Summary: This study describes the clinical characteristics of pathogenic variants in the *ACAN* gene and the response to rhGH treatment over three years in children with a heterozygous *ACAN* variant. The results indicate that rhGH therapy may be considered to reduce the height deficit in these patients, particularly in prepubertal children with ACAN deficiency.

Aggrecan is the major proteoglycan of the cartilage growth plate. Homozygous pathogenic *ACAN* variants lead to a severe form of skeletal dysplasia, whereas heterozygous *ACAN* variants can lead to milder phenotypes such as idiopathic short stature. *ACAN* variants can cause disproportionate short stature, advanced bone age and early-onset osteoarthritis. This study examined the genotype-phenotype correlations and assess the response to rhGH treatment in children with heterozygous *ACAN* variants.

36 children (23 boys, 13 girls) from the Dutch National Registry of GH treatment in children, with ACAN deficiency treated with rhGH for at least one year, were included in the analysis. 25 different heterozygous *ACAN* variants were identified in the 36 subjects. Most children showed features such as disproportionate growth, advanced bone age, early-onset osteoarthritis and dysmorphic features such as midface hypoplasia and brachydactyly. However, about 20% had no specific features. Children with truncating *ACAN* variants were shorter at the start of rhGH treatment than those with non-truncating variants. Prepubertal children showed a mean height gain of +0.6 SDS after 1 year and +1.0 SDS after 3 years of rhGH treatment. No significant increase in height gain was observed in pubertal children.

In conclusion, the phenotype associated with pathogenic heterozygous *ACAN* variants is highly variable. Genetic testing for ACAN deficiency is recommended in children with severe short stature, even in the absence of specific dysmorphic features. rhGH treatment may be considered in children with ACAN deficiency, but the variability in response highlights the need for personalized treatment approaches.

References

1. Domowicz MS, Cortes M, Henry JG, Schwartz NB. Aggrecan modulation of growth plate morphogenesis. *Dev Biol.* 2009; 329(2):242–57.
2. Nilsson O, Guo MH, Dunbar N, Popovic J, Flynn D, Jacobsen C, *et al*. Short stature, accelerated bone maturation, and early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol Metab.* 2014;99(8):E1510–8.
3. Gibson BG, Briggs MD. The aggrecanopathies; an evolving phenotypic spectrum of human genetic skeletal diseases. *Orphanet J Rare Dis.* 2016;11(1):86.
4. Muthuvel G, Dauber A, Alexandrou E, Ty- zinski L, Andrew M, Hwa V, *et al*. Treatment of short stature in aggrecan-deficient patients with recombinant human growth hormone: 1-year response. *J Clin Endocrinol Metab.* 2022;107(5):e2103–09.

# New Paradigms

## 4.7. IGF-1 acts through Kiss1-expressing cells to influence metabolism and reproduction

Wang M, Pugh SM, Daboul J, Miller D, Xu Y, Hill JW

*bioRxiv [Preprint].* 2024 Jul 4:2024.07.02.601722.
doi: 10.1101/2024.07.02.601722. PMID: 39005405

Brief Summary: IGF1R signaling in cells expressing Kiss1 gene affects energy balance, food intake, and physical activity in a sex-specific manner. Female IGF1R$^{Kiss1}$ mice showed lower body weight and food intake plus higher energy expenditure and physical activity. This phenotype was associated with higher proopiomelanocortin (POMC) expression. The additional deletion of insulin receptor (IR) in Kiss1-expressing cells reversed the lean phenotype seen in female IGF1R$^{Kiss1}$. In male mice, the loss of both IGF1R and IR signaling led to delayed puberty and reduced fertility. Overall, these results suggest cooperative roles of IGF1R and IR in metabolism and reproduction.

Kiss1-expressing neurons may link metabolic status to reproduction. Kisspeptin (encoded by Kiss1 gene) exerts its effects on the hypothalamic-pituitary-gonadal (HPG) axis by stimulating gonadotropin-releasing hormone (GnRH) neuron activity (1). Previous studies in rodents have shown an impact of nutrition on Kiss1 expression in the hypothalamus (2-4). IGF-1 and insulin act through related tyrosine kinase receptors and brain IGF-1 signaling has been shown to affect reproduction. Brain IGF1R knockout mice suffer from growth retardation and infertility, but deletion of IGF1R in GnRH neurons does not impact fertility. Based on these observations, the authors hypothesized that the central reproductive effects of IGF-1 may be mediated by IGF1R and IR signaling in Kiss1-expressing neurons.

To test these hypotheses, the authors characterized the metabolic and reproductive functions of mice lacking only IGF1R (IGF1R$^{Kiss1}$ mice) or both IGF1R and IR specifically in Kiss1-expressing cells (IGF1R/IR$^{Kiss1}$ mice).

Loss of IGF1R in Kiss1 cells in female mice caused lower body length and weight, lower food intake, higher energy expenditure and physical activity. This phenotype was linked to higher mRNA expression of proopiomelanocortin (POMC) in the hypothalamus. Male IGF1R$^{Kiss1}$ mice had only mild changes in metabolic profile. IGF1R/IR$^{Kiss1}$ female mice showed a reversed phenotype as compared to IGF1R$^{Kiss1}$ mice with higher fat mass, decreased hypothalamic mRNA expression of POMC and glucose intolerance, suggesting that IGF1R and IR signaling in Kiss1-expressing cells have divergent roles in regulating food intake and physical activity in female mice. This finding was only partially confirmed in IGF1R/IR$^{Kiss1}$ male mice.

Delayed puberty was found in both sexes as a result of single IGF1R deletion and combined IGF1R and IR deletions in Kiss1 cells. Male IGF1R$^{Kiss1}$ and IGF1R/IR$^{Kiss1}$ mice had impaired adulthood fertility in terms of reduced number of spermatids and spermatozoa in seminiferous tubules, and lower gonadotropin and testosterone levels.

This study elegantly uncovers another piece of the puzzle linking metabolism and reproduction, showing that IGF1R and IR have cooperative roles in regulating body size, metabolism and reproduction via Kiss1-expressing cells in a sex-specific manner.

References


1. Kirilov M, Clarkson J, Liu X, Roa J, Campos P, Porteous R, Schütz G, Herbison AE. Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. *Nat Commun.* 2013;4:2492. doi: 10.1038/ncomms3492. PMID: 24051579.
2. Luque RM, Kineman RD, Tena-Sempere M. Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. *Endocrinology.* 2007 Oct;148(10):4601-11. doi: 10.1210/en.2007-0500. Epub 2007 Jun 26. PMID: 17595226.
3. Castellano JM, Navarro VM, Fernández-Fernández R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology.* 2005 Sep;146(9):3917-25. doi: 10.1210/en.2005-0337. Epub 2005 Jun 2. PMID: 15932928.
4. Conde K, Kulyk D, Vanschaik A, Daisey S, Rojas C, Wiersielis K, Yasrebi A, Degroat TJ, Sun Y, Roepke TA. Deletion of Growth Hormone Secretagogue Receptor in Kisspeptin Neurons in Female Mice Blocks Diet-Induced Obesity. *Biomolecules.* 2022 Sep 25;12(10):1370. doi: 10.3390/biom12101370. PMID: 36291579; PMCID: PMC9599822.


## 4.8. Verapamil prevents decline of IGF-I in subjects with type 1 diabetes and promotes β-cell IGF-I signaling


Xu G, Chen J, Lu B, Sethupathy P, Qian WJ, Shalev A



*Diabetes*. 2023 Oct 1;72(10):1460-1469.
doi: 10.2337/db23-0256. PMID: 37494660

Brief Summary: This study examined the potential benefits of Verapamil, a calcium channel blocker traditionally used to treat hypertension, in Type 1 Diabetes (T1D). It shows that Verapamil can prevent the decline of Insulin-like Growth Factor I (IGF-I) levels, which are crucial for β-cell survival and insulin production. Moreover, Verapamil reduced the expression of IGF-binding protein 3 (IGFBP3), enhancing IGF-I signaling and protecting β-cells from apoptosis. Hence, Verapamil may be effective in preserving β-cells and improving glucose control in T1D patients.

Verapamil, an approved antihypertensive drug, has recently been proposed to have antidiabetic effects. Verapamil protects against β-cell death and diabetes in different mouse models of diabetes (1). Furthermore, a randomized controlled clinical trial with Verapamil showed an improvement of endogenous β-cell function with decreased insulin requirements in adults with recent-onset T1D (2). This preservation of β-cell function has also been further confirmed in a recent, independent Verapamil trial in children with T1D (3). IGF-1 and IGF-binding proteins are involved in insulin secretion and β-cell survival (4-6) and IGF-I levels are lower in subjects with T1D, correlating with β-cell function (7).

This study tested the hypothesis that the effects of Verapamil on β-cell function may be mediated by a modulation of the IGF system. Using serum samples of individuals with T1D before and after 1 year of treatment with Verapamil or placebo, and primary human and mouse islets as well as INS-1 cells, the authors found that Verapamil is able to preserve circulating IGF-I levels, downregulate islet IGFBPs, and promote IGF-I signaling.

To further explore the effects of verapamil on β-cell function and survival, experiments in human and mouse β-cells were conducted. Exposure to Verapamil reduced the expression of four of the six members of the IGFBP family. By reducing IGFBP-3 levels, Verapamil may thus enhance IGF-I bioavailability and signaling and contribute to the preservation of β-cell function. Finally, Verapamil decreased the expression of the proapoptotic thioredoxin-interacting protein (TXNIP) in human islets exposed to T1D-associated inflammatory cytokines. TXNIP promotes IGFBP-3 expression and inhibits the phosphorylation/activation of IGF1R.

In conclusion, the protective effect of Verapamil on β-cells may be partly mediated by the regulation of IGF-I signaling, which is crucial for β-cell survival and function.

References


1. Xu G, Chen J, Jing G, Shalev A. Preventing β-cell loss and diabetes with calcium channel blockers. *Diabetes.* 2012 Apr;61(4):848-56. doi: 10.2337/db11-0955. PMID: 22442301; PMCID: PMC3314354.
2. van Haeften TW, Twickler TB. Insulin-like growth factors and pancreas beta cells. *Eur J Clin Invest.* 2004 Apr;34(4):249-55. doi: 10.1111/j.1365-2362.2004.01337.x. PMID: 15086355.
3. Ovalle F, Grimes T, Xu G, Patel AJ, Grayson TB, Thielen LA, Li P, Shalev A. Verapamil and beta cell function in adults with recent-onset type 1 diabetes. *Nat Med.* 2018 Aug;24(8):1108-1112. doi: 10.1038/s41591-018-0089-4. Epub 2018 Jul 9. PMID: 29988125; PMCID: PMC6092963.
4. Forlenza GP, McVean J, Beck RW, Bauza C, Bailey R, Buckingham B, DiMeglio LA, Sherr JL, Clements M, Neyman A, Evans-Molina C, Sims EK, Messer LH, Ekhlaspour L, McDonough R, Van Name M, Rojas D, Beasley S, DuBose S, Kollman C, Moran A; CLVer Study Group. Effect of Verapamil on Pancreatic Beta Cell Function in Newly Diagnosed Pediatric Type 1 Diabetes: A Randomized Clinical Trial. *JAMA.* 2023 Mar 28;329(12):990-999. doi: 10.1001/jama.2023.2064. PMID: 36826844; PMCID: PMC9960020.
5. Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White MF, Herrera PL, Accili D, Efstratiadis A. Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. *J Clin Invest.* 2002 Oct;110(7):1011-9. doi: 10.1172/JCI15276. PMID: 12370279; PMCID: PMC151144.
6. Silha JV, Gui Y, Murphy LJ. Impaired glucose homeostasis in insulin-like growth factor-binding protein-3-transgenic mice. *Am J Physiol Endocrinol Metab.* 2002 Nov;283(5):E937-45. doi: 10.1152/ajpendo.00014.2002. PMID: 12376320.
7. Ekman B, Nyström F, Arnqvist HJ. Circulating IGF-I concentrations are low and not correlated to glycaemic control in adults with type 1 diabetes. *Eur J Endocrinol.* 2000 Oct;143(4):505-10. doi: 10.1530/eje.0.1430505. PMID: 11022197.
8. Palta M, LeCaire TJ, Sadek-Badawi M, Herrera VM, Danielson KK. The trajectory of IGF-1 across age and duration of type 1 diabetes. *Diabetes Metab Res Rev.* 2014 Nov;30(8):777-83. doi: 10.1002/dmrr.2554. PMID: 24845759; PMCID: PMC4236234.


## New Mechanisms

## 4.9. Reduction in pappalysin-2 levels and lower IGF-I bioavailability in female adolescents with anorexia nervosa


Barrios V, Martín-Rivada Á, Guerra-Cantera S, Campillo-Calatayud A, Camarneiro RA, Graell M, Chowen JA, Argente J

*J Clin Endocrinol Metab*. 2024 Feb 20;109(3):e920-e931.

doi: 10.1210/clinem/dgad713. PMID: 38066647

Brief Summary: This study investigated the effects of anorexia nervosa (AN) on the insulin-like growth factor (IGF) axis in female adolescents. It evaluated levels of various proteins and their role in the IGF axis, including pregnancy-associated plasma protein A2 (PAPP-A2) and stanniocalcins (STC-1 and STC-2). The reduced levels of PAPP-A2 may impair IGFBP cleavage, thereby reducing IGF-I bioavailability. IGF1 plays a crucial role in regulating growth, metabolism, and bone health.

This study assessed the impact of AN on IGF system function in 68 female adolescents diagnosed with AN and 62 healthy age-matched female controls. All participants had reached full pubertal maturity. Female adolescents with AN showed lower levels of both total and free IGF-I, a growth factor strongly influenced by nutritional status. A decrease in intact IGFBP-3 and an increase in intact IGFBP-4 were found in patients with AN, suggesting a change in IGF bioavailability and IGFBP cleavage. PAPP-A2, a metalloproteinase that cleaves IGFBPs to increase IGF bioavailability, was significantly reduced in AN patients. This reduction may contribute to lower IGF-I bioavailability, further exacerbating the effects of malnutrition. Furthermore, a significant correlation between reduced IGF-I and PAPP-A2 levels and reduced bone mineral density was found. In patients with secondary amenorrhea (a condition commonly seen in AN) lower IGF-I and IGFBP-3 levels were observed compared to those who retained menstrual cycles, indicating more severe endocrine disruptions in these individuals.

These findings highlight the dysregulation of IGF system in adolescents with AN, suggesting the need of monitoring the IGF system in the management of AN in adolescents. In particular, the assessment of PAPP-A2 levels could provide insight into how malnutrition affects bone metabolism and growth in patients with AN and could help develop targeted therapeutic strategies to limit these effects.

References


1. Silén Y, Keski-Rahkonen A. Worldwide prevalence of DSM-5 eating disorders among young people. *Curr Opin Psychiatry*. 2022;35(6): 362-371.
2. Muñoz MT, Argente J. Anorexia nervosa in female adolescents: endocrine and bone mineral density disturbances. *Eur J Endocrinol*. 2002;147(3):275-286.
3. Støving RK, Chen JW, Brixen K, Flyvbjerg A, Hørder K, Frystyk J. Bioactive insulin-like growth factor (IGF) I and IGF-binding protein-1 in anorexia nervosa. *J Clin Endocrinol Metab.* 2007;92(6):2323-2329.


## 4.10. WIP1 is a novel specific target for growth hormone action


Apaydin T, Zonis S, Zhou C, Valencia CW, Barrett R, Strous GJ, Mol JA, Chesnokova V, Melmed S
*iScience*. 2023 Oct 4;26(11):108117.
doi: 10.1016/j.isci.2023.108117. PMID: 37876819


Brief Summary: This study assessed the effect of growth hormone (GH) on WIP1 (wild-type p53-inducible phosphatase 1), a key mediator of the DNA damage response (DDR). GH suppressed DDR by inducing WIP1, which dephosphorylates and inactivates ATM (ataxia-telangiectasia mutated) kinase and its downstream effectors (such as CHK2, p53 and H2AX), leading to the accumulation of unrepaired DNA, which can potentially contribute to tumorigenesis.

This elegant study demonstrates that GH, through its receptor (GHR), is able to induce the production of WIP1, a phosphatase that dephosphorylates ATM and its effectors (CHK2, p53 and H2AX), in human colon cells and intestinal organoids. WIP1 was also elevated in patients with high GH levels due to somatotrophic adenomas, whereas it was decreased in GHR-deficient mice. Inhibition of WIP1 restored ATM phosphorylation and reversed DNA damage. A novel GH signalling pathway involving Src/AMPK and HIPK2 prevents WIP1 degradation. GH is able to induce WIP1 in human colon cells, intestinal organoids and mammary cells, leading to a reduction in ATM phosphorylation and subsequent accumulation of damaged DNA. GH activates the Src/AMPK pathway, which triggers the translocation of HIPK2 from the nucleus to the cytoplasm. This translocation reduces the ability of HIPK2 to ubiquitinate and degrade WIP1, thereby stabilising WIP1 and increasing its phosphatase activity.

These results suggest that GH-induced WIP1 activity could contribute to a pro-tumorigenic environment by enabling the accumulation of damaged DNA, particularly in epithelial cells. This mechanism may explain the increased risk of neoplasia observed in conditions associated with elevated GH levels, such as acromegaly. By identifying WIP1 as a mediator of the suppressive effects of GH on DDR, the study paves the way for new therapeutic interventions targeting the GH/WIP1 axis to prevent or treat certain types of cancer.

References

1. Schiewer, M.J., and Knudsen, K.E. (2016). Linking DNA Damage and Hormone Signaling Pathways in Cancer. *Trends Endocrinol. Metab.* 27, 216–225.
2. Chesnokova, V., and Melmed, S. (2020). Peptide Hormone Regulation of DNA Damage Responses. *Endocr. Rev.* 41, bnaa009.
3. Chesnokova, V., Zonis, S., Barrett, R., Kameda, H., Wawrowsky, K., Ben-Shlomo, A., Yamamoto, M., Gleeson, J., Bresee, C., Gorbunova, V., and Melmed, S. (2019). Excess growth hormone suppresses DNA damage repair in epithelial cells. *JCI Insight* 4, e125762.

## 4.11. Lower muscle protein synthesis in humans with obesity concurrent with lower expression of muscle IGF1 splice variants

Freitas EDS, Kras KA, Roust LR, De Filippis EA, Kimball SR, Buras M, Katsanos CS

*Obesity (Silver Spring)*. 2023 Nov;31(11):2689-2698.
doi: 10.1002/oby.23896. PMID: 37840435

Brief Summary: This cross-sectional study explored the relationship between obesity and skeletal muscle protein synthesis, focusing on the expression of IGF-I and its mRNA splice variants. It provides insights into how obesity might impair protein synthesis and IGF-I levels, potentially contributing to the reduced muscle quality observed in obese patients.

This study explored IGF-I expression in the muscles of obese patients as a potential factor contributing to their impaired muscle protein synthesis (1). It enrolled 9 adult volunteers with obesity and nine controls, matched for age and height. Muscle biopsy was performed to analyse mitochondrial function. Blood samples were collected for IGF-I, glucose, insulin and d9-leucine enrichment, that represents a marker of synthesis rates of muscle proteins (2,3). Obese patients showed lower mixed-muscle protein fractional synthesis rate compared to the controls, in addition to lower levels of mitochondrial protein synthesis. Obese patients had also lower expression of IGF-I and its receptor in their skeletal muscles compared to normal weight subjects. The expression of specific IGF-I mRNA splice variants, named IGF-I Eb and Ec, was lower in the muscle tissue of obese individuals. These splice variants are critical for muscle growth and repair and are closely related to mitochondrial protein synthesis in the skeletal muscle of healthy individuals but not in those with obesity (4).

These findings provide new insights into the molecular mechanisms underlying muscle dysfunction in obese subjects and highlight the need for further research to develop targeted interventions to mitigate these effects (5). The reduced expression of IGF-I suggests that therapeutic strategies aimed at increasing IGF-I signaling may improve muscle protein synthesis and overall muscle health in obese individuals.

References

1. Vassilakos G, Barton ER. Insulin-Like Growth Factor I Regulation and Its Actions in Skeletal Muscle. *Compr Physiol.* 2018 Dec 13;9(1):413-438. doi: 10.1002/cphy.c180010. PMID: 30549022.
2. Guillet C, Delcourt I, Rance M, Giraudet C, Walrand S, Bedu M, Duche P, Boirie Y. Changes in basal and insulin and amino acid response of whole body and skeletal muscle proteins in obese men. '*J Clin Endocrinol Metab.* 2009 Aug;94(8):3044-50. doi: 10.1210/jc.2008-2216. Epub 2009 May 26. PMID: 19470633.
3. Tran L, Masters H, Roust LR, Katsanos CS. A new method to measure muscle protein synthesis in humans by endogenously introduced d9-leucine and using blood for precursor enrichment determination. *Physiol Rep.* 2015 Aug;3(8):e12479. doi: 10.14814/phy2.12479. PMID: 26243214; PMCID: PMC4562565.
4. Brisson BK, Barton ER. Insulin-like growth factor-I E-peptide activity is dependent on the IGF-I receptor. *PLoS One.* 2012;7(9):e45588. doi: 10.1371/journal.pone.0045588. Epub 2012 Sep 21. PMID: 23029120; PMCID: PMC3448668.
5. O'Neill BT, Lauritzen HP, Hirshman MF, Smyth G, Goodyear LJ, Kahn CR. Differential Role of Insulin/IGF-1 Receptor Signaling in Muscle Growth and Glucose Homeostasis. *Cell Rep.* 2015 May 26;11(8):1220-35. doi: 10.1016/j.celrep.2015.04.037. Epub 2015 May 14. PMID: 25981038; PMCID: PMC4449334.

## 4.12. Metabolic surgery-induced changes of the growth hormone system relate to improved adipose tissue function

Gancheva S, Kahl S, Herder C, Strassburger K, Sarabhai T, Pafili K, Szendroedi J, Schlensak M, Roden M

*Int J Obes (Lond)*. 2023 Jun;47(6):505-511.
doi: 10.1038/s41366-023-01292-7. PMID: 36959287

Brief Summary: This longitudinal study explored the impact of metabolic surgery on the growth hormone (GH)/IGF-1 axis and its subsequent effects on adipose tissue function. The results suggest that bariatric surgery enhances GH-IGF-1 levels, which are linked to improved adipose tissue function and a reduction in inflammation markers.

This study explored how metabolic surgery influences the GH system, and how these changes are related to improvements in adipose tissue function. Markers of glucose metabolism, adipose tissue function, leptin levels, IGF-I, GH, and inflammatory status were assessed in 79 obese male adults before and after bariatric surgery, compared to 24 matched non-obese controls. As predicted, at baseline, obese patients had higher levels of serum insulin, leptin, and IGFBP3, similar levels of IGF-1, and lower levels of GH and IGFBP1 compared to controls. By 2 weeks post-surgery, there was an increase in serum GH and IGFBP1 levels and this effect improved throughout the follow-up period of 52 weeks, reaching levels that were higher than or equal to those of the controls. IGF-I levels began to increase later, with significant rises observed at 24- and 52-weeks post-surgery. No significant differences were found between participants who underwent sleeve gastrectomy and those who had gastric bypass surgery. The restoration of the GH/IGF-I system was linked to improvements in insulin sensitivity in adipose tissue, and to a reduction in insulin levels, free fatty acids levels and inflammation markers (IL1-ra, reactive C protein, TGFβ1).

These findings suggest that normalization of GH and IGF-I secretion in adipose tissue is a key mechanism by which surgery improves metabolic health (1,2).

References


1. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet.* 2002 May 18;359(9319):1740-5. doi: 10.1016/S0140-6736(02)08655-5. PMID: 12049864.
2. Faramia J, Hao Z, Mumphrey MB, Townsend RL, Miard S, Carreau AM, Nadeau M, Frisch F, Baraboi ED, Grenier-Larouche T, Noll C, Li M, Biertho L, Marceau S, Hould FS, Lebel S, Morrison CD, Münzberg H, Richard D, Carpentier AC, Tchernof A, Berthoud HR, Picard F. IGFBP-2 partly mediates the early metabolic improvements caused by bariatric surgery. *Cell Rep Med.* 2021 Apr 20;2(4):100248. doi: 10.1016/j.xcrm.2021.100248. PMID: 33948578; PMCID: PMC8080239.


## New Perspectives

## 4.13. Saturation genome editing of *BAP1* functionally classifies somatic and germline variants


Waters AJ, Brendler-Spaeth T, Smith D, Offord V, Tan HK, Zhao Y, Obolenski S, Nielsen M, van Doorn R, Murphy JE, Gupta P, Rowlands CF, Hanson H, Delage E, Thomas M, Radford EJ, Gerety SS, Turnbull C, Perry JRB, Hurles ME, Adams DJ



*Nat Genet*. 2024 Jul;56(7):1434-1445.
doi: 10.1038/s41588-024-01799-3. PMID: 38969833


Brief Summary: These authors performed exhaustive saturation genome editing (SGE) of *BAP1* (BRCA1-associated protein 1), the disruption of which is linked to tumorigenesis and altered neurodevelopment. 18,108 unique variants were characterized, of which 6,196 were found to have abnormal functions. These were then used to evaluate phenotypic associations in the UK Biobank. *BAP1* variants were also characterized in a large population-ascertained tumor collection, in cancer pedigrees and ClinVar. Disruptive germline *BAP1* variants were associated with higher circulating levels of the mitogen IGF-1, suggesting a possible pathological mechanism and a therapeutic target.

BAP1 is a widely expressed deubiquitinating enzyme that plays a critical role in several cellular processes, including transcriptional regulation, cell cycle, response to DNA damage and regulation of chromatin dynamics. Germline *BAP1* variants were associated with increased levels of IGF-1. This finding suggests a possible pathogenic mechanism involving a close cross-talk between BAP1 and IGF system and suggests potential therapeutic targets.

This study functionally characterised 18,108 unique *BAP1* variants, achieving near-complete coverage of the possible variants of the gene. This extensive and detailed mapping is unprecedented in genetic research. The

authors developed a variant classifier based on the SGE data with over 98% sensitivity and specificity. This represents a significant advance in the interpretation of genetic variants, particularly those categorised as "variants of uncertain significance" (VUS), which pose challenges in clinical practice. A significant difference in the effect of different *BAP1* variants was observed, particularly between those associated with cancer and those associated with neurodevelopmental disorders.

Disruptive germline *BAP1* variants are associated with increased levels of IGF-1, a mitogen that promotes tumour growth. This suggests a possible pathogenic mechanism and highlights potential therapeutic targets. The use of SGE allowed a more precise differentiation between variants that contribute to cancer and those associated with neurodevelopmental disorders.

These innovative findings significantly advance our understanding of BAP1's role in diseases and provide advanced tools for precision medicine, enabling more accurate prediction of disease risk and guiding therapeutic decisions.

References


1. Landrum, M. J. *et al*. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* 46, D1062–D1067 (2018).
2. Carbone, M. *et al*. BAP1 and cancer. Nat. Rev. Cancer 13, 153–159 (2013).
3. Nishikawa, H. *et al*. BRCA1-associated protein 1 interferes with BRCA1/BARD1 RING heterodimer activity. *Cancer Res.* 69, 111–119 (2009).
4. Pollak, M. N., Schernhammer, E. S. & Hankinson, S. E. Insulin-like growth factors and neoplasia. *Nat. Rev. Cancer* 4, 505–518 (2004).


## 4.14. Evaluation of the *MC3R* gene pertaining to body weight and height regulation and puberty development


Zheng Y, Rajcsanyi LS, Peters T, Dempfle A, Wudy SA, Hebebrand J, Hinney A

*Sci Rep.* 2023 Jun 27;13(1):10419.

doi: 10.1038/s41598-023-37344-1. PMID: 37369769


Brief Summary: This study examined the role of the melanocortin 3 receptor (*MC3R*) gene in regulating body weight, height, and puberty timing. It found an association between various non-synonymous variants (NSVs) in the *MC3R* gene with energy homeostasis and puberty.

Melanocortin 3 receptor (MC3R) is a G protein-coupled receptor involved in the hypothalamic system that regulates energy homeostasis, growth and puberty (1,2). Recent studies in *MC3R*-knockout mice showed an altered body weight regulation, severe insulin resistance and lipid profile alterations (3,4). In this study, Sanger sequencing of the coding region of *MC3R* was performed in 185 children or adolescents with short normal stature (SNS) or 258 individuals with severe obesity, and 192 healthy-lean individuals. 11 variants (6 NSVs) were identified. Notably, 3 rare loss-of-function (LoF) variants (p.Phe45Ser, p.Arg220Ser, and p.Ile298Ser) were exclusively found in severely obese individuals, suggesting a potential link between these variants and severe obesity. Conversely, the novel NSV p.Ala214Val, predicted to increase protein stability, was identified in a single lean female. In-silico analyses performed for all detected variants, suggested that four missense variants, including p.Ala214Val, may have pathogenic effects, with p.Ala214Val potentially increasing protein stability. The interaction network for MC3R, generated using GeneMANIA, identified 20 genes linked to BMI, height, and puberty timing.

In conclusion, the study underscores the importance of MC3R in body weight regulation, growth, and puberty timing. The identification of specific *MC3R* variants in obese individuals and in children with SNS suggests that variants in this gene may contribute to these conditions (5).

References


1. Hinney A, Volckmar AL, Antel J. Genes and the hypothalamic control of metabolism in humans. *Best Pract Res Clin Endocrinol Metab.* 2014 Oct;28(5):635-47. doi: 10.1016/j.beem.2014.04.007. Epub 2014 Apr 26. PMID: 25256760.. 28, 635–647 (2014).
2. Lam BYH, Williamson A, Finer S, Day FR, Tadross JA, Gonçalves Soares A, Wade K, Sweeney P, Bedenbaugh MN, Porter DT, Melvin A, Ellacott KLJ, Lippert RN, Buller S, Rosmaninho-Salgado J, Dowsett GKC, Ridley KE, Xu Z, Cimino I, Rimmington D, Rainbow K, Duckett K, Holmqvist S, Khan A, Dai X, Bochukova EG; Genes & Health Research Team; Trembath RC, Martin HC, Coll AP, Rowitch DH, Wareham NJ, van Heel DA, Timpson N, Simerly RB, Ong KK, Cone RD, Langenberg C, Perry JRB, Yeo GS, O'Rahilly S. MC3R

links nutritional state to childhood growth and the timing of puberty. *Nature.* 2021 Nov;599(7885):436-441. doi: 10.1038/s41586-021-04088-9. Epub 2021 Nov 3. PMID: 34732894; PMCID: PMC8819628.
3. Butler AA, Cone RD. Knockout studies defining different roles for melanocortin receptors in energy homeostasis. *Ann N Y Acad Sci.* 2003 Jun;994:240-5. doi: 10.1111/j.1749-6632.2003.tb03186.x. PMID: 12851322.
4. You P, Hu H, Chen Y, Zhao Y, Yang Y, Wang T, Xing R, Shao Y, Zhang W, Li D, Chen H, Liu M. Effects of Melanocortin 3 and 4 Receptor Deficiency on Energy Homeostasis in Rats. *Sci Rep.* 2016 Oct 7;6:34938. doi: 10.1038/srep34938. PMID: 27713523; PMCID: PMC5054679.
5. Renquist BJ, Lippert RN, Sebag JA, Ellacott KL, Cone RD. Physiological roles of the melanocortin MC3 receptor. *Eur J Pharmacol.* 2011 Jun 11;660(1):13-20. doi: 10.1016/j.ejphar.2010.12.025. Epub 2011 Jan 3. PMID: 21211527; PMCID: PMC3095771.

# 5. Bone, Growth Plate and Mineral Metabolism

Thomas Edouard[1], Anju Jacob[2]

[1]Endocrine, Bone Diseases and Genetics Unit, Reference Centre for Rare Diseases of Calcium and Phosphate Metabolism, OSCAR Network, ERN BOND, Children's Hospital, Toulouse University Hospital; RESTORE, INSERM U1301, Paul Sabatier University; Toulouse, France [2]Al Jalila Children's Specialty Hospital Mohammed Bin Rashid University of Health sciences Dubai Health, Dubai, UAE

## Advances in Clinical Practice

### 5.1. Hypophosphatasia diagnosis: current state of the art and proposed diagnostic criteria for children and adults

Aliya A. Khan, Maria Luisa Brandi, Eric T. Rush, Dalal S. Ali, Hatim Al-Alwani, Khulod Almonaei, Farah Alsarraf et al
*Osteoporosis International:* 2024; 35(3): 431–38.
doi: 10.1007/s00198-023-06844-1

In brief: This systematic review, conducted by an international group of experts, provides an overview of the phenotype of patients with hypophosphatasia and proposes updated diagnostic criteria for this disease in children and adults.

Commentary: Hypophosphatasia (HPP) is a rare genetic disorder caused by loss-of-function mutations in the alkaline phosphatase (ALPL) gene, which encodes the tissue nonspecific alkaline phosphatase (TNSALP) enzyme. Low alkaline phosphatase activity is associated with impaired mineralisation of the skeleton, manifested by signs of rickets and osteomalacia, as well as extraskeletal manifestations (such as failure to thrive, seizures, muscle weakness, gross motor delay, abnormal gait, pain, early loss of primary teeth, and other dental problems).

Diagnosis of HPP is based on a combination of clinical and radiographic signs, laboratory profile and the identification of a pathogenic or likely pathogenic variant in the *ALPL* gene. Its presentation can be extremely variable and the diagnosis is often delayed, in both children and adults, with a median time to diagnosis of 5.7 years from the onset of first signs and symptoms. During this time, individuals may experience complications of the disease and may also be misdiagnosed and/or mistreated with drugs such as bisphosphonates, which may further impair the underlying skeletal mineralisation defect. Therefore, it is important to establish precise diagnostic criteria to allow early diagnosis and appropriate evaluation and treatment of the multisystem complications of HPP. In addition, an accurate description of the disease is important to identify phenotype-genotype correlations.

### 5.2. Clinical profiles of children with hypophosphatasia prior to treatment with enzyme replacement therapy: an observational analysis from the global HPP registry

Gabriel Ángel Martos-Moreno, Cheryl Rockman-Greenberg, Keiichi Ozono, Anna Petryk, Priya S. Kishnani, Kathryn M. Dahir, Lothar Seefried, Shona Fang, Wolfgang Högler, Agnès Linglart
*Hormone Research in Paediatrics* 2024; 97 (3): 233–42.
doi: 10.1159/000531865

In brief: This observational analysis, based on data from the Global Hypophosphatasia Registry, reports the clinical profiles, prior to initiation of asfotase alfa enzyme replacement therapy, in a large cohort of children with hypophosphatasia (n = 151), by age (< 6 months vs 6 months to 18 years) and geographic region (USA/Canada, Europe, and Japan).

Commentary: Hypophosphatasia (HPP) is a rare genetic disorder caused by loss-of-function mutations in the alkaline phosphatase (ALPL) gene, which encodes the tissue nonspecific alkaline phosphatase (TNSALP) enzyme. Low alkaline phosphatase activity is associated with impaired mineralisation of the skeleton, manifested by signs of rickets and osteomalacia, as well as extraskeletal manifestations (such as failure to thrive, seizures, muscle weakness, gross motor delay, abnormal gait, pain, early loss of primary teeth, and other dental problems). Clinical manifestations vary widely, especially with age, with perinatal and infantile forms being the most severe and potentially life-threatening.

Enzyme replacement therapy with asfotase alfa has led to improvements in the various manifestations of the disease and is now approved in the US/Canada and Europe. However, there are no global treatment guidelines for the initiation of asfotase alfa, and recommendations for the initiation of this treatment may vary from country to country. Given the wide clinical variability of the disease, a precise description of the signs and symptoms of HPP is needed to specify the indication and effects of treatment with asfotase alfa.

This observational study provides a precise description of the frequency of the various manifestations, both skeletal and extraskeletal, particularly as a function of age. In particular, it is reported that children with HPP are more likely to have skeletal, renal and respiratory manifestations at first presentation at $<6$ months of age, whereas dental, muscular and pain manifestations are more common in those aged 6 months to 18 years at first presentation. Evaluation and follow-up of clinical data may shed light on the emergence of associations between phenotypic features, the regional differences, and the impact of enzyme replacement therapy on the natural history of HPP, including treatment outcomes that are relevant to routine clinical practice.

## 5.3. The Global ALPL gene variant classification project: dedicated to deciphering variants

Mariam R Farman, Catherine Rehder, Theodora Malli, Cheryl Rockman-Greenberg, Kathryn Dahir, Gabriel Ángel Martos-Moreno, Agnès Linglart, et al.
*Bone* 2024; 178:116947.
doi: 10.1016/j.bone.2023.116947

In brief: This article describes the functionalities of the Global *ALPL* gene variant classification project, which aims to reclassify variants of uncertain significance (VUS) in the *ALPL* gene and to continuously assess and update genetic, phenotypic, and functional variant information in hypophosphatasia.

Commentary: Hypophosphatasia (HPP) is a rare genetic disorder caused by loss-of-function mutations in the alkaline phosphatase (*ALPL*) gene, which encodes the tissue nonspecific alkaline phosphatase (TNSALP) enzyme. Low alkaline phosphatase activity is associated with impaired mineralisation of the skeleton, manifested by signs of rickets and osteomalacia, as well as extraskeletal manifestations (such as failure to thrive, seizures, muscle weakness, gross motor delay, abnormal gait, pain, early loss of primary teeth, and other dental problems).

Diagnosis of the disease is based on a combination of clinical and radiographic signs, laboratory profile and the identification of a pathogenic or likely pathogenic variant in the *ALPL* gene. Analysis of the *ALPL* gene is therefore an important step in the diagnostic process, but variants of uncertain significance (VUS) can cause diagnostic delay and uncertainty for patients and healthcare providers.

In 2021, the Global *ALPL* Gene Variant Classification Project was established to reclassify VUS in the *ALPL* gene and to continuously assess and update genetic, phenotypic, and functional variant information in hypophosphatasia. To this end, the database provides a submission system for clinicians, geneticists, genetic counselors, and researchers to submit VUS within *ALPL* for classification. An international, multidisciplinary consortium of HPP experts was established to reclassify the submitted VUS using a multi-step process including a clinical phenotype assessment, in-depth literature review, molecular genetic assessment, and in-vitro functional testing of variants in a cotransfection model to measure ALP residual activity.

This classification project will help to define the milder range of the disease, but also to characterise new and existing HPP phenotypes, leading to improved care for these patients.

## 5.4. Serum phosphorus as a driver of skeletal morbidity in fibrous dysplasia

Zubeyir Hasan Gun, Charles Osamor, Jocelyn Taylor, Xiaobai Li, Vivian Szymczuk, Alison M. Boyce
*Journal of Clinical Endocrinology and Metabolism* 2024; 109(5): 1334 40.
doi: 10.1210/clinem/dgad671

In brief: This retrospective study reports on the relationship between serum phosphorus and fibrous dysplasia-related skeletal complications (fractures, orthopaedic surgery, and scoliosis) in a large cohort of patients (n = 240).

Commentary: Fibrous dysplasia (FD) / McCune-Albright syndrome (MAS) is a rare mosaic disorder caused by postzygotic gain-of-function mutations in Gαs, resulting in continuous receptor activation. FD can range from an isolated, asymptomatic monostotic lesion to severe polyostotic disease associated with an increased risk of fracture, deformity, functional impairment, and pain. FD lesions are related to proliferation of abnormally differentiated osteoprogenitor cells and replacement of normal bone and marrow with expansile fibro-osseous tissue. Abnormal osteoprogenitor cells overproduce FGF23, resulting in hyperphosphaturia with or without hypophosphatemia. Several studies have reported that patients with hypophosphatemia are at increased risk of FD-related complications, suggesting that dysplastic, poorly mineralised FD tissue may be particularly vulnerable to the effects of low phosphorus levels. The current study demonstrates that both, frank hypophosphatemia (Z-score $\leq -2$) and low-normophosphatemia ($> -2$ to $\leq -1$), are associated with increased skeletal complications in patients with FD. These findings confirm FGF23 excess as an important contributor to skeletal morbidity, and provide support for a robust approach to monitoring and treatment.

Studies of FGF23 excess disorders, such as X-linked hypophosphatemia (XLH), have shown that burosumab, a monoclonal antibody to FGF23, can robustly and safely correct serum phosphorus into the normal range. A phase 2 trial of burosumab in patients with FD is ongoing (NCT05509595); based on the results reported here, it has been designed to target high-normophosphatemia and will titrate burosumab to achieve phosphorus levels between Z-score $>$ -1 and $\leq$ 2. The results of that trial will hopefully improve our understanding of the safety and feasibility of therapeutically targeting high normal phosphorus levels.

## 5.5. The IMPACT survey: a mixed methods study to understand the experience of children, adolescents and adults with osteogenesis imperfecta and their caregivers

Westerheim, Ingunn, Tracy Hart, Taco van Welzenis, Lena Lande Wekre, Oliver Semler, Cathleen Raggio, Michael B. Bober, Maria Rapoport, Samantha Prince, Frank Rauch
*Orphanet Journal of Rare Diseases* 20241; 9(1): 128.
doi: 10.1186/s13023-024-03126-9

In brief: The IMPACT Survey collected a comprehensive dataset (including demographics, clinical characteristics and clinical signs, symptoms and events and their impact) on the experience of individuals with osteogenesis imperfecta (OI) (n = 2312 individuals across self- and proxy reports), and their caregivers (n = 560). Individuals with OI reported numerous and evolving symptoms that affect their quality of life, notably pain and fatigue, which are consistently present.

Commentary: Osteogenesis imperfecta (OI) is a rare genetic skeletal disorder mainly caused by pathogenic variants in the type 1 collagen gene that results in bone fragility with fractures, bone deformities, and short stature. Patients may also present with extraosseous manifestations, including dentinogenesis imperfecta, hearing loss, joint hypermobility, blue sclerae, and cardiac/respiratory defects. Currently, treatment is mainly symptomatic, as part of a multidisciplinary approach to promote mobility and limit pain, fractures and bone deformities. Given the multi-systemic and chronic nature of the disease, there is a significant impact on quality of life, in terms of the physical, emotional and psychological aspects for individuals with OI, as well as their families and caregivers. However, to date, data on health-related quality of life are incomplete, relate to small cohorts and usually do not assess the impact on their families and caregivers.
The value of this survey is to provide a comprehensive dataset on the experience of individuals with OI and their caregivers. This has been achieved through an ambitious and self-driven collaboration between the clinical and

OI communities. Upcoming analyses will provide further insights into the economic impact, healthcare journey and caregiver wellbeing, with the aim of contributing to improved treatment and care for the OI community. This survey may serve as a model for other rare genetic diseases.

## 5.6. A practical guide to the diagnosis and management of osteoporosis in childhood and adolescence

Leanne M. Ward

*Frontiers in Endocrinology.* 2023; 14:1266986.
doi: 10.3389/fendo.2023.1266986

In Brief: This up-to-date and very clear review describes the diagnosis and management of primary or secondary bone fragility in children and adolescents.

Commentary: The field of bone fragility in children has evolved considerably in recent years, particularly in terms of diagnosis and management. This progress has been made possible in particular by the discovery of new genes involved in childhood-onset bone fragility, and also by a better understanding of the natural history of bone fragility secondary to acute or chronic pathology.

This review provides a step-by-step guide to the diagnosis and management of children presenting with fragility fractures. It also addresses unmet needs, particularly the need for an expanded toolbox of effective osteoporosis agents in children, including anabolic agents. In the case of secondary bone fragility, an important point highlighted in this review is the potential for resolution of bone fragility in growing children once the risk factors have been removed (e.g. recovery from acute leukaemia). After the diagnostic phase, assessing this potential for spontaneous healing is a key step in determining the need for drug treatment to increase bone mass. In terms of therapeutic options, there is currently virtually only one treatment available: bisphosphonates, which have the potential to inhibit bone resorption. Although the efficacy and tolerability of bisphosphonates in primary and secondary bone fragility have been widely reported, one of the challenges for the future is to develop new treatments, particularly those with an anabolic effect on bone.

# Novel Treatments

## 5.7. Burosumab vs conventional therapy in children with x-linked hypophosphatemia: results of the open-label, phase 3 extension period

Leanne M. Ward, Wolfgang Högler, Francis H. Glorieux, Anthony A. Portale, Michael P. Whyte, Craig F. Munns, Ola Nilsson, et al.

*JBMR Plus* 2024; 8(1): ziad001.
doi: 10.1093/jbmrpl/ziad001

In brief: This report describes the efficacy and safety of burosumab during the open-label extension period of the original Phase 3 study (weeks 64-88) in 21 children with X-linked hypophosphatemia (XLH) who continued to receive burosumab or crossed over from conventional therapy to burosumab.

Commentary: X-linked hypophosphatemia (XLH) is a rare inherited disorder of phosphorus metabolism caused by loss-of-function mutations in the *PHEX* gene, resulting in excessive plasma levels of a phosphate-wasting hormone called FGF23. Increased circulating FGF23 inhibits renal phosphate reabsorption and 1,25-dihydroxyvitamin D synthesis, resulting in rickets and osteomalacia, manifested mainly by short stature, bowing of the legs, gait abnormalities and impaired physical function.

Traditionally, XLH has been treated with multiple daily doses of phosphate and an active form of vitamin D. Burosumab is a novel monoclonal antibody to FGF23 approved for the treatment of XLH. In an original randomised, open-label phase 3 study in 61 children aged 1-12 years with XLH previously treated with conventional therapy, switching to burosumab every 2 weeks for 64 weeks restored normal phosphate levels,

cured rickets and improved growth and leg bowing compared to conventional therapy. In the current open-label extension phase (weeks 64-88), 21 children originally treated with burosumab or conventional therapy for 64 weeks were followed up to 88 weeks. Children who remained on burosumab continued to show improvements in rickets from baseline between weeks 64 and 88. Children who crossed over from conventional therapy to burosumab showed rapid improvement in phosphate metabolism and improved rickets resolution, confirming the findings of the 64-week pivotal trial. As in the original 64-week study, burosumab was well tolerated in children who continued burosumab for 88 weeks and in those who crossed over from conventional therapy.

These data provide additional support for the use of burosumab in the treatment of paediatric XLH.

## 5.8. Management of RANKL-mediated disorders with denosumab in children and adolescents: a global expert guidance document

Joel A. Vanderniet, Vivian Szymczuk, Wolfgang Högler, Signe S. Beck-Nielsen, Suma Uday, Nadia Merchant, Janet L. Crane, Leanne M. Ward, Alison M. Boyce, Craig F. Munns

*Journal of Clinical Endocrinology and Metabolism* 2024; 109(5): 1371–82.
doi: 10.1210/clinem/dgad657

In brief: This article reviews the evidence and provides expert opinion on the safe and appropriate use of denosumab in children and adolescents with RANKL-mediated disorders such as giant cell bone tumours, fibrous dysplasia and juvenile Paget's disease.

Commentary: Receptor activator of nuclear factor κB ligand (RANKL) is expressed by osteogenic cells and induces osteoclast differentiation by binding to RANK on osteoclast precursors. Excessive production of RANKL, as seen in RANKL-mediated bone tumours, results in focal bone destruction or systemic unregulated bone turnover. Various therapies have been used to treat these disorders (including surgery and medical treatments such as bisphosphonates) with variable efficacy and generally high occurence rates.

Denosumab is a humanised monoclonal antibody that inhibits RANKL with potent anti-osteoclastic effects. Although efficacy has been demonstrated in large prospective studies in adults with RANKL-mediated bone tumours, there is limited experience with the use of denosumab in children and adolescents due to the rarity of these disorders. Published data suggest that denosumab is effective in reducing osteoclastic activity and the expansion of focal lesions in RANKL-mediated bone tumours and fibrous dysplasia, with subsequent ossification with continued treatment. However, the rate of lesion recurrence after treatment cessation is important, with some lesions requiring subsequent surgery or otherwise long-term denosumab treatment. In addition, rebound hypercalcemia between doses or after discontinuation of denosumab is common in children and adolescents and appears to be greater than in adults.

These authors provide expert opinion on the safe and appropriate use of denosumab in children and adolescents with RANKL-mediated disorders. It highlights the need for patients to be managed in a tertiary specialist centre by a multidisciplinary team with expertise in managing denosumab treatment and the rebound phenomenon. Expert recommendations are given on the indication for denosumab treatment, its implementation and monitoring (efficacy and safety). The authors also discuss the use of intravenous bisphosphonates to prevent rebound hypercalcaemia after discontinuation of denosumab or during dose or interval tapering. Further collaborative research is needed to determine optimal treatment regimens and minimise risks.

## 5.9. Safety and efficacy of denosumab in children with osteogenesis imperfecta-the first prospective comparative study

Jiayi Liu, Xiaoyun Lin, Lei Sun, Qian Zhang, Yan Jiang, Ou Wang, Xiaoping Xing, Weibo Xia, Mei Li

*Journal of Clinical Endocrinology and Metabolism* 2024; 109(7): 1827–36.
doi: 10.1210/clinem/dgad732

In brief: This 1-year, open-label, randomised controlled trial examined the effects and tolerability of denosumab, compared with zoledronic acid, on bone mineral density (BMD), spinal morphometry, and safety in a large cohort of children (n = 84) with osteogenesis imperfecta. Treatment with denosumab increased BMD and improved spinal morphometry in children with OI, but was frequently associated with rebound hypercalcaemia (in 31%). This rebound hypercalcaemia could be alleviated by switching to zoledronic acid treatment.

Commentary: Osteogenesis imperfecta (OI) is a rare genetic skeletal disorder mainly caused by pathogenic variants in the type 1 collagen gene that results in bone fragility with fractures, bone deformities, and short stature. Patients may also present with extraosseous manifestations, including dentinogenesis imperfecta, hearing loss, joint hypermobility, blue sclerae, and cardiac/respiratory defects. To date, intravenous bisphosphonates, which inhibit bone resorption by promoting osteoclast apoptosis, are virtually the only treatment available for children with OI. However, it has been reported that bisphosphonates have poor efficacy in specific types of OI with different pathophysiology (e.g. OI type 5). Therefore, new antiresorptive drugs, such as denosumab, and anabolic agents need to be investigated in patients with bone fragility.

Denosumab, a humanised monoclonal antibody targeting receptor activator of nuclear factor κB ligand (RANKL), has been approved for the treatment of primary osteoporosis in adult patients. By binding to RANKL, a key mediator of osteoclast differentiation, function, and survival, denosumab works through a different pathway from bisphosphonates to increase BMD and reduce the fracture incidence in adult patients with osteoporosis. The current study showed that denosumab also significantly increased BMD and improved the spinal morphometry in children with OI. Regarding the tolerability, as previously reported, rebound hypercalcaemia was a quite common adverse event of denosumab in children with OI (31% of whom 46% even developed hypercalcaemic crisis) with an average of 4.7 months since the last denosumab injection. This rebound hypercalcaemia could be alleviated by switching to zoledronic acid treatment.

Treatment with denosumab appears to be a promising new option in the treatment of bone fragility in children. Further studies are needed to confirm its efficacy and to determine optimal treatment regimens and minimise risks.

## 5.10. Vosoritide therapy in children with achondroplasia aged 3-59 months: a multinational, randomised, double-blind, placebo-controlled, phase 2 trial

Ravi Savarirayan, William R. Wilcox, Paul Harmatz, John Phillips, Lynda E. Polgreen, Louise Tofts, Keiichi Ozono, et al.
*Lancet Child & Adolescent Health* 2024; 8(1): 40–50.
doi: 10.1016/S2352-4642(23)00265-1

In brief: This multinational, randomised, double-blind, placebo-controlled, Phase 2 study examined the safety and efficacy of vosoritide, a recombinant C-type natriuretic peptide (CNP) analogue, in 75 children with achondroplasia under age 5 years. Mean gain in height Z-score after 52-weeks was 0•25 (95% CI -0•02 to 0•53). The study was sponsored by BioMarin Pharmaceutical.

Commentary: Achondroplasia is one of the most common constitutional bone disorders (> 300,000 affected individuals worldwide). It is associated with skeletal defects (disproportionate short stature, abnormal curvature of the spine, genu varum) as well as extra-skeletal manifestations (reduction of the upper airway with otitis and sleep apnoea, stenosis of the foramen magnum and lumbar canal with spinal cord compression, hypotonia and muscle weakness, overweight and obesity) with the potential for functional limitations and psychosocial challenges. Achondroplasia is caused by a gain-of-function mutation in the fibroblast growth factor receptor 3 (FGFR3) gene, which leads to increased activation of the RAS/mitogen-activated protein kinase (MAPK) pathway in chondrocytes, resulting in impaired endochondral ossification. C-type natriuretic peptide (CNP) upon binding its receptor on chondrocytes has the property to inhibit the RAS/MAPK pathway. Vosoritide, a recombinant CNP analogue, has been reported to increase annualised growth velocity in children with achondroplasia aged 5-18 years.

In this Phase 2 study in infants and children under 5 years of age, vosoritide showed with no serious treatment-related adverse events and resulted in improved height Z-scores. Interestingly, in addition to the effects on growth, treatment with vosoritide was also associated with changes in facial volume, sinus volume and foramen

magnum area. These data, which need to be validated in a future phase 3 trial, are very promising as one of the challenges of early treatment of these children, in addition to improving growth, would be to mitigate other complications of achondroplasia (in particular sleep apnoea, stenosis of the foramen magnum, hypotonia and muscle weakness).

Various therapeutic trials are currently being evaluated in achondroplasia (vosoritide, TransCon CNP, infigratinib). It will be important to determine the relative efficacy of these different treatments in the future, and also to evaluate therapies combining these compounds.

## 5.11. Vosoritide treatment for children with hypochondroplasia: a phase 2 trial

Andrew Dauber, Anqing Zhang, Roopa Kanakatti Shankar, Kimberly Boucher, Tara McCarthy, Niusha Shafaei, Raheem Seaforth, Meryll Grace Castro, Niti Dham, Nadia Merchant

*EClinicalMedicine* 2024; 71:102591.
doi: 10.1016/j.eclinm.2024.102591

In brief: This single-arm, open-label, single-centre, Phase 2 study in the US examined the safety and efficacy of vosoritide, a recombinant C-type natriuretic peptide (CNP) analogue, in 26 children with hypochondroplasia. Height SDS increased by (mean) 0.36 during the 12-month treatment period versus the observation period. The study was sponsored by BioMarin Pharmaceutical.

Commentary: Hypochondroplasia is a rare skeletal dysplasia that manifests mainly as disproportionate short stature and relative macrocephaly, and occasionally mild orthopaedic manifestations such as tibial bowing and limited elbow extension. As achondroplasia, hypochondroplasia is caused by a gain-of-function mutation in the fibroblast growth factor receptor 3 (FGFR3) gene, which leads to increased activation of the RAS/mitogen-activated protein kinase (MAPK) pathway in chondrocytes, resulting in impaired endochondral ossification and short stature. Individuals with hypochondroplasia have different genetic variants in the *FGFR3* gene than those seen in individuals with achondroplasia, although their clinical spectrum can overlap, with hypochondroplasia generally being less severe. Vosoritide is a recombinant C-type natriuretic peptide (CNP) analogue that has been reported to increase annualised growth velocity in children with achondroplasia; this compound is now approved for the treatment of children with achondroplasia in the US/Canada and Europe. Given the pathophysiological mechanisms shared with achondroplasia (activation of the RAS/ MAPK pathway), it was logical to evaluate the efficacy of vosoritide in hypochondroplasia.

This study provides preliminary evidence of the efficacy of vosoritide in improving growth in children with hypochondroplasia with a relatively benign side effect profile. Further studies are needed to determine the long-term effects of vosoritide in children with hypochondroplasia.

## 5.12. Once-weekly transCon CNP (navepegritide) in children with achondroplasia (ACcomplisH): a phase 2, multicentre, randomised, double-Blind, placebo-controlled, dose-escalation trial

Ravi Savarirayan, Daniel G. Hoernschemeyer, Merete Ljungberg, Yuri A. Zarate, Carlos A. Bacino, Michael B. Bober, Janet M. Legare, et al.

*EClinicalMedicine* 2023; 65:102258.
doi: 10.1016/j.eclinm.2023.102258

In brief: This multinational, randomised, double-blind, placebo-controlled, dose-escalation Phase 2 study evaluated the safety and efficacy of TransCon CNP (navepegritide), a C-type natriuretic peptide (CNP) analogue designed to allow continuous CNP exposure with once-weekly dosing, in 42 children with achondroplasia aged 2-10 years. The study was sponsored by Ascendis Pharma.

Commentary: Achondroplasia is one of the most common constitutional bone disorders ($>$ 300,000 affected individuals worldwide). It is associated with skeletal defects (disproportionate short stature, abnormal curvature

of the spine, genu varum) but also with extra-skeletal manifestations (reduction of the upper airway with otitis and apnoea, stenosis of the foramen magnum and lumbar canal with spinal cord compression, hypotonia and muscle weakness) with the potential for functional limitations and psychosocial challenges. Achondroplasia is caused by a gain-of-function mutation in the fibroblast growth factor receptor 3 (FGFR3) gene, which leads to increased activation of the RAS/mitogen-activated protein kinase (MAPK) pathway in chondrocytes, resulting in impaired endochondral ossification.

Transcon-CNP is a long-acting prodrug that releases native CNP and has demonstrated beneficial effects on bone growth in both mouse and non-human primate models. Results from a Phase 1 study showed that TransCon CNP was well tolerated in healthy adult volunteers. In this Phase 2 study in children with achondroplasia aged 2-10 years, TransCon CNP was well tolerated with no serious treatment-related adverse events and demonstrated a dose-dependent improvement in annualised growth velocity compared to placebo.

This study provides the first evidence that TransCon CNP increases annualised growth velocity in children with achondroplasia with a favourable safety profile, supporting further investigation of its safety and efficacy, as well as its impact on achondroplasia-related health complications.

Various therapeutic trials are currently being evaluated in achondroplasia (vosoritide, TransCon CNP, infigratinib). It will be important to determine the relative efficacy of these different treatments in the future, and also to evaluate therapies combining these compounds.

## 5.13. Low-dose infigratinib increases bone growth and corrects growth plate abnormalities in an achondroplasia mouse model

Benoit Demuynck, Justine Flipo, Nabil Kaci, Carl Dambkowski, Morgan Paull, Elena Muslimova, Bhavik P. Shah, Laurence Legeai-Mallet

*Journal of Bone and Mineral Research*. 2024; zjae051.
doi: 10.1093/jbmr/zjae051

In brief: This study investigated the effect of infigratinib, a selective and orally bioavailable FGFR1-3 inhibitor, administered at different doses or according to different dosing regimens, on bone growth in a mouse model mimicking achondroplasia ($Fgfr3^{Y367C/+}$). This study was partially sponsored by a grant from BridgeBio/QED Therapeutics.

Commentary: Achondroplasia is one of the most common constitutional bone diseases (> 300,000 affected individuals worldwide). It is associated with skeletal defects (disproportionate short stature, abnormal curvature of the spine, genu varum) but also with extra-skeletal manifestations (reduction of the upper airway with otitis and apnoea, stenosis of the foramen magnum and lumbar canal with spinal cord compression, hypotonia and muscle weakness) with the potential for functional limitations and psychosocial challenges. Achondroplasia is caused by a gain-of-function mutation in the fibroblast growth factor receptor 3 (FGFR3) gene, resulting in impaired endochondral ossification.

Infigratinib is an orally bioavailable, selective FGFR tyrosine kinase inhibitor being developed for the treatment of FGFR-driven cancers such as cholangiocarcinoma and urothelial carcinoma. Using a mouse model mimicking achondroplasia, it was previously reported that daily treatment with infigratinib at a dose of 2 mg/kg significantly increased bone growth. In the present study, lower doses (0.2 and 0.5 mg/kg, given once daily) were sufficient to observe significant improvements in axial and appendicular skeletal growth and skull development. In addition, analyses showed an increase in the area of the foramen magnum at the base of the skull, improving foramen magnum stenosis, a well-recognised complication of achondroplasia. This proof-of-concept study shows that low-dose infigratinib has the potential to be a safe and effective treatment option for children with achondroplasia.

Phase 2 and 3 clinical trials are currently underway to test the efficacy of infigratinib in children with acondroplasia. Recently announced results of a Phase 2 study of infigratinib in children with achondroplasia demonstrated a significant and robust increase in annualized height velocity, with a mean change of 3.38 cm/year from baseline, and no treatment–related adverse effects [1].

Various therapeutic trials are currently being evaluated in achondroplasia (vosoritide, TransCon CNP, infigratinib). It will be important to determine the relative efficacy of these different treatments in the future, and also to evaluate therapies combining these compounds.

Reference

1. Savarirayan R, De Bergua JM, Arundel P, et al. OR27-03 oral Infigratinib treatment is well tolerated and significantly increases height velocity in children with achondroplasia: month 6 results from the PROPEL 2 dose finding study. *J Endocr Soc*. 2023;7(Supplement_1):A814. doi: https://doi.org/10.1210/jendso/bvad114.1525

## Advances in Growth, Bone Biology and Mineral Metabolism

### 5.14. Modeling human skeletal development using human pluripotent stem cells

Shireen R. Lamandé, Elizabeth S. Ng, Trevor L Cameron, Louise H. W. Kung, Lisa Sampurno, Lynn Rowley, Jinia Lilianty, et al.
*Proceedings of the National Academy of Sciences of the USA*. 2023; 120(19): e2211510120.
doi: 10.1073/pnas.2211510120

In brief. This basic research study used an *in vitro* method to recapitulate key steps in growth plate development and endochondral bone formation, using induced pluripotent stem cells (iPSCs). This method provides original material that can be used to study growth plate and bone development, to assess the consequences of genetic variants involved in bone diseases, and to test new therapies.

Commentary: In vertebrates, the longitudinal growth of long bones is determined through endochondral ossification at the growth plate level. During this process, chondrocytes, which are derived from the condensation of undifferentiated mesenchymal cells, undergo a series of proliferation and differentiation steps. First, small chondrocytes rapidly divide and form columns in the proliferating zone. Then, in the pre- and early-hypertrophic zone, chondrocytes undergo hypertrophic differentiation, characterised by increased cell size and the synthesis of collagen type 10, which is then calcified by enzymes such as alkaline phosphatase. In the late hypertrophic zone, terminally differentiated chondrocytes undergo apoptosis, and the cartilaginous calcified matrix is degraded, through the simultaneous action of matrix metalloproteinase and the invasion of blood vessels, along with osteoclasts, and replaced by bone.

These authors report an *in vitro* method that recapitulates the various proliferation and differentiation steps of endochondral bone formation. Using induced pluripotent stem cells (iPSCs), this method allows the generation of cartilage organoids that can be directed towards an articular phenotype or a growth plate phenotype that matures to hypertrophy. The hypertrophic chondrocytes of the growth plate can in turn be differentiated into osteoblasts that synthesise the bone-specific extracellular matrix and mineralise this matrix. A detailed analysis of the gene expression profile during the different stages of proliferation and differentiation is provided.

This method provides original material for studying growth plate and bone development, assessing the consequences of genetic variants involved in bone diseases and testing new therapies.

### 5.15. Osteocyte mitochondria regulate angiogenesis of transcortical vessels

Peng Liao, Long Chen, Hao Zhou, Jiong Mei, Ziming Chen, Bingqi Wang, Jerry Q. Feng, et al.
*Nature Communications* 2024; 15(1): 2529.
doi: 10.1038/s41467-024-46095-0

In brief: This basic research article provides new insights into osteocyte-transcortical vessel interactions and potentially opens up new therapeutic perspectives for bone diseases associated with vascular damage.

Commentary: The bone vascular system has been shown to play a critical role in controlling bone development and wound healing and provides a microenvironment for the differentiation and maturation of haematopoietic and immune cells in bone marrow. In particular, capillaries that originate in the bone marrow and cross the cortical bone, named transcortical vessels (TCVs), appear to play an essential role in communication between

the bone marrow vascular system and the external circulation. Although TCVs occupy over 86% of the cortical bone canals, the regulatory factors that determine the formation and maintenance of TCVs are still unknown. Osteocytes, the major cell component of cortical bone, are in close contact with endothelial cells of TCVs. Therefore, it has been hypothesised that osteocytes may act as a critical mediator in the regulation of the TCV homeostasis.

This study shows that osteocytes maintain a normal TCV network by transferring mitochondria to endothelial cells in cortical bone. Partial ablation of osteocytes causes TCV regression and reduction of angiogenic genes. Inhibition of mitochondrial transfer by conditional knockout of Rhot1 in osteocytes also leads to regression of the TCV network. In contrast, MIRO1-mediated mitochondrial transfer from osteocytes to endothelial cells rescued endothelial dysfunction. Metabolomic analysis further revealed that mitochondrial transfer induces the biosynthesis and SPHK1-dependent catalysis of D-sphingosine. Administration of D-sphingosine promotes TCVs and bone formation in mouse cortical bone defect model.

These findings reveal a unique mechanism involved in the vascular homeostasis of cortical bone and open new therapeutic perspectives for bone diseases associated with vascular damage.

# 6. Differences of Sexual Development (DSD) and Gender Incongruence (GI)

Tulay Guran[1], Sabine E Hannema[2], Busra Gurpinar Tosun[3]

[1]Pediatric Endocrinology and Diabetes, Marmara University, School of Medicine, Istanbul, Turkey *tulay.guran@-marmara.edu.tr, tulayguran@yahoo.com* [2]Department of Pediatric Endocrinology, Amsterdam UMC location Vrije Universiteit Amsterdam Amsterdam, The Netherlands. *s.e.hannema@amsterdamumc.nl* [3]Pediatric Endocrinology and Diabetes, Marmara University, School of Medicine, Istanbul, Turkey *gurpinarbusra@gmail.com*

## Preface

In the past 12 months, between July 2023 and 2024, the search for "Differences of Sexual Development" or "disorders of sex development" or "ambiguous genitalia" or "gonadal development" or "DSD" and "gender incongruence" or "gender dysphoria" in PubMed yielded more than 1000 publications published in English. Among those, 15 are summarized in this chapter. The selection process was very challenging given the limited space. We prioritized key publications chosen on the quality of methodology, the significance of the outcome, and particularly the impact on clinical practice. We endeavored to balance basic research and clinical articles.

This year's selected articles on disorders of sex development (DSD) cover topics such as 1. Novel genes and mechanisms involved in gonadal development, 2. Further clinical and molecular insights into SF1 deficiency, and Klinefelter syndrome. The selected articles on gender incongruence present new clinical insights on the effects of the use of GnRH analogues and gender-affirming sex hormone therapy on psychological health, fertility, bone health and cardiovascular health outcomes in transgender individuals.

We hope these selected publications will help with understanding and improve both knowledge and the clinical care of patients.

## DSD - Novel Genes and Mechanisms involved in Gonadal Development

### 6.1. The -KTS splice variant of *WT1* is essential for ovarian determination in mice

Gregoire EP, De Cian MC, Migale R, Perea-Gomez A, Schaub S, Bellido-Carreras N, Stévant I, Mayère C, Neirijnck Y, Loubat A, Rivaud P, Sopena ML, Lachambre S, Linssen MM, Hohenstein P, Lovell-Badge R, Nef S, Chalmel F, Schedl A, Chaboissier MC *Science*. 2023 Nov 3;382(6670):600-606.
doi: 10.1126/science.add8831. PMID: 37917714

Brief summary: This study examined the complex process of sex determination in mice, with a specific focus on the role of *WT1* isoforms, +KTS and -KTS, during gonadal development. The study provides insights into how the balance between these isoforms impacts sexual differentiation, with broader implications for understanding gonadal dysgenesis conditions, like Frasier syndrome.

While the role of the *SRY* gene in testicular determination is well understood, the factors influencing ovarian determination are less clear. This study highlights the crucial role of the -KTS isoform in female development and suggests that sex determination depends not only on the presence of key factors like Sry for males and -KTS for females but also on the precise timing of their expression.

A key player in early gonadal development is the Wilms' tumor suppressor (WT1), a zinc finger transcriptional regulator located on chromosome 11p13. *WT1* produces two major isoforms through alternative splicing: +KTS, which includes three amino acids (KTS) between the last two zinc fingers,

and -KTS, which excludes them. Mutations in the donor splice site in intron 9 of *WT1*, which cause Frasier syndrome, lead to an imbalance favoring the -KTS isoform over +KTS, resulting in 46,XY gonadal dysgenesis.

This study examined the gonads of -KTS and +KTS knockout mouse models using transcriptomic analyses. They found that the absence of +KTS is compensated by an increase in -KTS expression, and that this increase of -KTS rather than the loss of +KTS, is involved in the pathogenesis of gonadal dysgenesis in Frasier syndrome. Their results indicate that elevated -KTS expression prevents *Sry* upregulation, promotes pre-granulosa cell differentiation, and triggers premature ovarian differentiation, disrupting testicular development in XY mice. The study also underscores the essential role of the -KTS isoform in initiating ovarian development in XX embryos and maintaining Sertoli cell differentiation in XY embryos after Sry expression.

Although direct comparisons between mouse and human data are challenging due to differences in the timing and dynamics of sex determination, the findings suggest that the -KTS isoform of *WT1* is crucial for gonadal development and is essential for initiating ovarian differentiation.

## 6.2. Variants in *SART3* cause a spliceosomopathy characterised by failure of testis development and neuronal defects

Ayers KL, Eggers S, Rollo BN, Smith KR, Davidson NM, Siddall NA, Zhao L, Bowles J, Weiss K, Zanni G, Burglen L, Ben-Shachar S, Rosensaft J, Raas-Rothschild A, Jørgensen A, Schittenhelm RB, Huang C, Robevska G, van den Bergen J, Casagranda F, Cyza J, Pachernegg S, Wright DK, Bahlo M, Oshlack A, O'Brien TJ, Kwan P, Koopman P, Hime GR, Girard N, Hoffmann C, Shilon Y, Zung A, Bertini E, Milh M, Ben Rhouma B, Belguith N, Bashamboo A, McElreavey K, Banne E, Weintrob N, BenZeev B, Sinclair AH

*Nat Commun*. 2023 Jun 9;14(1):3403.
doi: 10.1038/s41467-023-39040-0. PMID: 37296101

Brief summary: This translational study reveals a novel mechanism underlying syndromic gonadal dysgenesis (GD). It introduces a condition termed INDYGON syndrome (Intellectual disability, **N**eurodevelopmental defects and **D**evelopmental delay with 46,X**Y GON**adal dysgenesis).

46,XY gonadal dysgenesis (GD) is a rare disorder of sex development (DSD) affecting 1-9 per 100,000 live births. Genetic diagnoses are achieved in less than half of cases despite advanced techniques. Many patients exhibit additional cardiac, renal, skeletal, and neurological abnormalities, classifying them as syndromic GD. Early molecular diagnosis is essential for timely detection and management of GD and associated comorbidities.

This multicenter study identified 9 individuals with biallelic "Squamous cell carcinoma antigen recognized by T cells 3" (*SART3*) gene mutations. Five had 46,XY complete or partial GD, while the four 46,XX individuals had typical female genitalia and no reproductive disorders. All affected individuals exhibited neurodevelopmental defects, intellectual disability, hypotonia, craniofacial anomalies, corpus callosal agenesis or hypoplasia, ventriculomegaly, and cerebellar anomalies.

The spliceosome, essential for splicing non-coding introns from precursor mRNA, depends on the SART3 protein for recycling small nuclear RNAs (snRNAs). Ayers KL et al. demonstrated SART3's conserved role in gonadal and neural development using a Drosophila model and testis-like organoids derived from human induced pluripotent stem cells (iPSCs) with patient-specific SART3 variants. In the RNAi-mediated SART3 orthologue knockdown Drosophila model, male flies were infertile, with disrupted spermatocytes, spermatids, and somatic cells, leading to immature, misshaped testes. Neuronal-specific knockdown caused complete embryonic lethality due to impaired neuronal development and midline defects.

Testis-like organoids homozygous for one of the human *SART3* variants associated with XY GD were smaller with increased apoptosis. Transcriptomic analysis of *SART3* variant iPSCs revealed alternative transcript usage in over 300 genes and widespread gene expression changes, including upregulation of many spliceosome components.

While further long-term studies are necessary to fully understand how gene-splicing abnormalities contribute to this condition, the identification of INDYGON syndrome underscores the critical role of spliceosome components like SART3 in both testicular and brain development.

## 6.3. Complete male-to-female sex reversal in XY mice lacking the miR-17~92 cluster

Hurtado A, Mota-Gómez I, Lao M, Real FM, Jedamzick J, Burgos M, Lupiáñez DG, Jiménez R, Barrionuevo FJ
*Nat Commun*. 2024 May 7;15(1):3809.
doi: 10.1038/s41467-024-47658-x. PMID: 38714644

Brief summary: This molecular study explored the role of microRNAs (miRNAs) in mammalian sex determination. It used bulk and single-cell RNA-seq (scRNA-seq) alongside time-course expression analyses in a knockout mouse model to assess the role of the miR-17~92 cluster in this process.

miRNAs are small, single-stranded, non-coding RNA molecules, 21 to 23 nucleotides in length, that mediate post-transcriptional gene regulation. Hurtado A et al. demonstrated that deleting the miR-17~92 cluster induces complete gonadal dysgenesis in XY mice. The miR-17~92 cluster, known as OncomiR-1, is a polycistronic cluster containing six miRNA genes (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1) involved in various developmental and pathogenic processes. Members of this cluster are expressed in mouse gonads of both sexes during and after the sex determination stage. Additionally, these miRNAs are evolutionarily conserved and linked to sex development and sex change in non-mammalian vertebrates.

These authors used the Cre/LoxP system to generate XY knockout (KO) mice lacking the miR-17~92 cluster ($Tg(CAG\text{- }cre)^{1Nagy}$;$miR\text{-}17\sim92^{del/del}$). XY KO mice developed as phenotypic females with two uterine horns and ovaries. Histological analysis of XY KO gonads revealed absence of Sertoli cells at the sex determination stage and a clear ovarian morphology. Sry expression was delayed in XY KO gonads. Double immunofluorescence analysis showed that XY KO gonads did not express the testicular marker Sox9 but instead expressed the ovarian marker Foxl2.

Bulk RNA-seq on gonads revealed that both XY and XX mutants acquired a female-like expression profile, with most genes displaying an ovarian-specific expression pattern. Furthermore, molecular pathways involved in cell proliferation and growth control (e.g., Ras protein signal transduction, MAP kinase activity, ERK1/ERK2 cascade) were altered. Consistently, miR-17~92 KO gonads were ~50% smaller than wild-type controls. Analysis of proliferation rates in gonads showed that the genital ridge of mutant gonads contained fewer proliferating progenitor cells than controls.

This study highlights, for the first time, the role of miR-17~92 in controlling early gonadal growth and Sry expression dynamics, underlines the importance of non-coding genome elements such as miRNAs in sex determination and adds complexity to our understanding of this process.

## 6.4. Microdeletion at *ESR1* intron 6 (DEL_6_75504) is a susceptibility factor for cryptorchidism and hypospadias

Masunaga Y, Fujisawa Y, Massart F, Spinelli C, Kojima Y, Mizuno K, Hayashi Y, Sasagawa I, Yoshida R, Kato F, Fukami M, Kamatani N, Saitsu H, Ogata T
*J Clin Endocrinol Metab*. 2023 Sep 18;108(10):2550-2560.
doi: 10.1210/clinem/dgad187. PMID: 37010083

Brief summary: This translational study identifies a new susceptibility region within the estrogen receptor α (*ESR1*) gene, linked to cryptorchidism (CO) and hypospadias (HS), the most common birth defects of the male genital tract. CO occurs in approximately 1:100 male births and is 3-times more common in premature infants, while HS affects about 1:300 male births. The prevalence of both conditions has increased over the years, with premature infants showing higher prevalence and severity.

CO and HS are influenced by various genetic and environmental factors, including specific single-gene mutations. Masunaga Y et al. previously identified a tight linkage disequilibrium (LD) block within *ESR1*, spanning 5 single nucleotide polymorphisms (SNPs) in Japanese boys with CO and HS. Four major haplotypes were identified, with the "AGATC" haplotype being strongly associated with both conditions in a recessive manner. The researchers suggested that the "AGATC" haplotype could enhance estrogenic effects through increased *ESR1* expression, contributing to CO and HS development.

In this study, 230 Italian boys (80 with CO and 150 with typical male genitalia) and 415 Japanese boys (149 with CO, 141 with HS, and 125 with typical male genitalia) were enrolled to investigate the susceptibility factor linked to this haplotype. Using Sanger sequencing, SNP typing, and whole-genome sequencing (WGS) methods, a 2244-bp microdeletion within the *ESR1* gene, termed ΔESR1 (Structural variant: DEL_6_75504), was identified as the true susceptibility factor for CO and HS. This conclusion is based on the positive association of ΔESR1 with CO and HS and its nearly absolute LD with the "AGATC" haplotype in both Japanese and Italian boys, along with the observed upregulation of *ESR1* expression linked to ΔESR1.

Expression studies using MCF-7 cells demonstrated that ΔESR1 leads to increased *ESR1* expression, particularly under estrogenic conditions, which may explain its role in enhancing susceptibility to CO and HS, especially when exposed to environmental endocrine disruptors. These findings shed new light on the genetic basis of CO and HS, emphasizing the importance of ΔESR1 in the pathogenesis of these conditions. However, further investigation is needed on the frequency of this susceptibility locus in different populations, its effects on HS and CO development, and the availability of tests to explore the causal relationship between ΔESR1 and other risk loci in individuals with CO and HS.

## Clinical and Molecular Insights into SF1 Deficiency

### 6.5. Clinical and genetic characteristics of a large international cohort of individuals with rare *NR5A1* /SF-1 variants of sex development

Kouri C, Sommer G, Martinez de Lapiscina I, Elzenaty RN, Tack LJW, Cools M, Ahmed SF, Flück CE; SF1next study group
*EBioMedicine*. 2024 Jan;99:104941.
doi: 10.1016/j.ebiom.2023.104941. PMID: 38168586

Brief summary: The SF1next study describes a cohort of 197 individuals with *NR5A1/SF-1* variants, identified through the I-DSD registry and a research network involving 55 centers across 18 countries. *NR5A1/SF-1* plays a crucial role in the development and function of human sex and steroid producing organs, and variants in this gene can significantly affect early sex determination and differentiation. This can lead to a wide spectrum of differences in sex development (DSD), from healthy carriers to severe forms of DSD. Despite extensive research, a comprehensive understanding of the diverse clinical presentations of *NR5A1/SF-1* variants is still lacking.

SF1next is a global collaborative effort aimed at collecting data on the largest cohort to date, to address the variability of rare DSD associated with *NR5A1/SF-1* variants. The study used a standardized dataset consisting of data collected during care delivered according to international recommendations for the optimal clinical care of individuals with DSD. The findings confirmed a broad range of phenotypes, from severe DSD (defined as $>2$ of the genital features degree of labioscrotal fusion, length of the genital tubercle, position of the urethral meatus, and locations of the right and left gonads being atypical for karyotype prior to any surgery) in over 70% of 46,XY individuals to healthy carriers in 90% of 46,XX females. Phenotypic variability was observed even among families with the same genetic variants, indicating no clear genotype–phenotype correlation. The study concludes that phenotypic variability in *NR5A1/SF-1* variants may involve additional genetic factors, suggesting a possible oligogenic cause for some individuals with DSD.

The authors acknowledge the limitations of relying on data not originally collected for the specific purpose of this study and sometimes partially incomplete or inaccurate data due to problems inherent to the study design and use of registry data in this multicenter retrospective study.

Beyond genetics and DSD phenotype, information on birth weight, anthropometric measurements at the last visit, involvement of other organ systems, surgical procedures performed on patients, germ cell tumor development, and pubertal developmental course were included. Collectively, these results expand our knowledge of individuals with *NR5A1/SF-1* variants.

## 6.6. A conserved NR5A1-responsive enhancer regulates *SRY* in testis-determination

Houzelstein D, Eozenou C, Lagos CF, Elzaiat M, Bignon-Topalovic J, Gonzalez I, Laville V, Schlick L, Wankanit S, Madon P, Kirtane J, Athalye A, Buonocore F, Bigou S, Conway GS, Bohl D, Achermann JC, Bashamboo A, McElreavey K

*Nat Commun*. 2024 Mar 30;15(1):2796.

doi: 10.1038/s41467-024-47162-2. PMID: 38555298

Brief summary: This study identified a conserved enhancer element located 5' of the mammalian *SRY* gene through comparative genomic analysis, which plays a crucial role in the regulation of sexual differentiation. NR5A1 binds to this element. The researchers discovered two distinct hemizygous base pair substitutions within this NR5A1 binding site, both of which involve highly conserved residues: one in a sporadic case of XY sex reversal and the other in a large familial case of Y-linked 46,XY gonadal dysgenesis (1).

Despite the discovery of *SRY* over 30 years ago, the mechanisms that regulate its expression during testis determination remain poorly understood. In this study, the researchers first identified a conserved NR5A1-binding element in the open chromatin region upstream of the *SRY* gene. This 250bp long DNA region, designated E250, was accessible in vivo at the time of human testis determination, as shown by DNase-seq analysis of human fetal testis and single-cell ATAC-seq data from somatic cells in the human embryonic XY gonad. It was hypothesized that variants within this conserved region might influence human testis determination and development. To test this hypothesis, the team sequenced DNA from 358 individuals with unexplained 46,XY gonadal dysgenesis using Sanger sequencing or whole-genome sequencing. Two unique sequence variants were identified in the *SRY* 5' flanking region, both located within the proposed E250 NR5A1-binding site.

In silico modeling predicted that single nucleotide substitutions disrupted the interaction between the NR5A1 protein and its corresponding response element. Further, a protocol differentiating human-induced pluripotent stem cells into gonadal progenitors (2) demonstrated that a deletion of the NR5A1 binding site in E250 was associated with a significant reduction of *SRY* expression during the critical phase of SRY induction.

The results of this comprehensive study underscore the importance of pathogenic variants in the regulatory elements of disease-causing genes in contributing to the etiology of 46,XY DSD. This study identified a novel cause for 46,XY DSD linked to *SRY* and proposed that the exquisite sensitivity to time- and dose-dependent thresholds might explain the phenotypic variability observed in familial cases.

References

1. Kirtane, J. & Kaddu, S. 46XY disorder of sexual differentiation in five generations: a preliminary report. *J. Prog. Paediatr. Urol.* 17,75–79 (2014).
2. Gonen N, Eozenou C, Mitter R, et al. In vitro cellular reprogramming to model gonad development and its disorders. *Sci Adv*. 2023;9(1):eabn9793. doi: 10.1126/sciadv.abn9793.

## 6.7. Long-read genome sequencing reveals a novel intronic retroelement insertion in *NR5A1* associated with 46,XY differences of sexual development

Del Gobbo GF, Wang X, Couse M, Mackay L, Goldsmith C, Marshall AE, Liang Y, Lambert C, Zhang S, Dhillon H, Fanslow C, Rowell WJ, Care4Rare Canada Consortium, Marshall CR, Kernohan KD, Boycott KM

*Am J Med Genet A*. 2024 May;194(5):e63522.

doi: 10.1002/ajmg.a.63522. PMID: 38131126

Brief summary: This study performed long-read genome sequencing (lr-GS) using PacBio HiFi on several members of a four-generation family presenting with autosomal dominant (AD) 46,XY differences of sexual

development (DSD). This family had undergone a lengthy molecular diagnostic process with no conclusive results. Lr-GS offers enhanced mapping capabilities in highly repetitive, homologous, and low-complexity regions, providing better assessment of structural variations and complex genomic rearrangements compared to short-read genome sequencing (sr-GS).

The advent of sr-GS has significantly improved the detection of rare genetic disorders by extending sequencing beyond the exome, identifying non-coding and structural variations. While it is challenging to quantify the diagnostic yield improvement over exome sequencing, it may be as high as 10%. Lr-GS, which produces reads that are 100–1000 times longer, addresses many of sr-GS's limitations, offering a more comprehensive genomic view (1, 2).

In this reported family, some individuals exhibited incomplete penetrance, with at least one unaffected 46,XY carrier. Affected members displayed various genital phenotypes, including hypospadias, ambiguous genitalia, dysgenetic testes, and reduced fertility. DNA from 4 affected participants across 2 generations was sequenced using the Sequel IIe system (PacBio), generating HiFi long reads. Lr-GS identified a novel SINE-VNTR-Alu (SVA) element insertion in intron 4 of *NR5A1*, linked to haploinsufficiency, a known mechanism in AD 46,XY DSD. This was missed b y sr-GS although it was called by 1 of the 3 structural variant tools in 3 of 5 affected individuals, due to the filtering criteria. Functional studies using RT-qPCR and RNA-Seq confirmed that the SVA insertion is associated with reduced *NR5A1* expression, although differential expression could not be conclusively confirmed due to the lack of appropriate samples from controls.

This study highlights that retroelement insertions are likely an underestimated cause of unexplained rare genetic disorders. The utility of lr-GS as a promising tool for studying unexplained rare diseases is demonstrated, emphasizing the role of undiscovered non-coding variations in Mendelian disorders.

References

1. Chaisson MJP, Sanders AD, Zhao X, et al. Multiplatform discovery of haplotype-resolved structural variations in human genomes. *Nat Commun.* 2019;10(1):1784. Published 2019 Apr 16. doi: 10.1038/s41467-018-08148-z
2. Logsdon GA, Vollger MR, Eichler EE. Long-read Human Genome Sequencing and Applications. *Nat Rev Genet.* 2020;21(10):597-614. doi: 10.1038/s41576-020-0236-x

## New Clinical Insights into Klinefelter Syndrome

### 6.8. Testicular dysfunction in 47,XXY boys: when it all begins. a semilongitudinal study

Pozza C, Sesti F, Tenuta M, Spaziani M, Tarantino C, Carlomagno F, Minnetti M, Pofi R, Paparella R, Lenzi A, Radicioni A, Isidori AM, Tarani L, Gianfrilli D

*J Clin Endocrinol Metab*. 2023 Sep 18;108(10):2486-2499.
doi: 10.1210/clinem/dgad205. PMID: 37043499

Brief summary: This longitudinal study provides an in-depth analysis of the clinical, endocrine, and testicular ultrasound (US) patterns in Klinefelter Syndrome (KS) from infancy through puberty and into adulthood, highlighting the progression of testicular degeneration in these patients.

In this study, 155 KS patients with the classical 47,XXY karyotype, aged 7 months to 55 years, were prospectively evaluated at a single referral center following a standardized protocol. Key findings were:

- Hormonal indicators of Sertoli and germ cell impairment emerge during Tanner stages 3 to 4. INHB levels remain normal until stage 4, after which a decrease in the INHB/FSH ratio is observed.
- FSH levels begin to rise at Tanner stage 2, more than LH levels. Although nearly all adult patients develop a hypergonadotropic state with compensated hypoandrogenism, impaired testosterone secretion is generally not seen in boys with KS during puberty.
- A reduced plasma testosterone/LH (T/LH) ratio may indicate Leydig cell dysfunction, even if plasma testosterone levels appear normal. The T/LH ratio peaks during Tanner stages 2 to 3, then declines from stage 4 onward. Testosterone levels increase until Tanner stage 4, then begin to regress.

- There is no hormonal evidence of testicular dysfunction (in terms of gonadotropins, testosterone and inhibin B) between mini-puberty and late prepuberty.
- Testicular volume increases from Tanner stage 2 to stage 4, with testicular degeneration becoming evident at the end of puberty (stage 5) and continuing into adulthood.
- Quantitative ultrasound measures, including echotexture and the presence of hypoechoic lesions and microlithiasis, are significant predictors of poor testicular endocrine function and they worsen through puberty and into the transition age, indicating ongoing gonadal compromise.

Further research is needed to assess the effectiveness of testicular ultrasound (US) in predicting outcomes for KS patients such as the chance of successful sperm retrieval and the optimal timing for therapeutic interventions. Integrating US findings with histological analysis and advanced hormone assays should help in optimizing patient care and improving long-term outcomes.

## 6.9. Executive dysfunction in Klinefelter syndrome: associations with brain activation and testicular failure

Foland-Ross LC, Ghasemi E, Lozano Wun V, Aye T, Kowal K, Ross J, Reiss AL
*J Clin Endocrinol Metab*. 2023 Dec 21;109(1):e88-e95.
doi: 10.1210/clinem/dgad487. PMID: 37595261

Brief summary: This clinical cross-sectional study provides new data on the neurodevelopmental aspects of Klinefelter syndrome (KS). It investigated the brain activation patterns in 43 adolescent males with KS (mean age 12.3 ± 2.3 years) compared to an age-matched typically-developing (n = 41) control group during the go/no-go task, an executive function assessment. Behavioral assessment tests, and functional magnetic resonance imaging (fMRI) were used.

Adolescents with KS showed reduced task accuracy and lower activation in brain regions involved in executive function, such as the right inferior frontal gyrus, anterior insula, striatum, and dorsal anterior cingulate gyrus. In the KS group, reduced activation in the paracingulate and dorsal anterior cingulate cortex was linked to lower testosterone levels and smaller testes volume, suggesting a relationship between pubertal development and brain function. Parent-reported executive dysfunction also correlated with lower testes volume but go/no go task performance did not.

These findings indicate a neural basis for executive dysfunction in KS, in which altered pubertal development and testosterone deficiency may play a role, although the study design does not allow causal inference. Reduced testicular volume and reduced brain activation might also both be signs of a more severe phenotype overall.

Future studies should further explore whether executive dysfunction in KS can be partially reversed with testosterone replacement therapy (TRT) and whether the timing of TRT initiation affects outcomes.

## 6.10. Detection of chromosomal aneuploidy in ancient genomes

Anastasiadou K, Silva M, Booth T, Speidel L, Audsley T, Barrington C, Buckberry J, Fernandes D, Ford B, Gibson M, Gilardet A, Glocke I, Keefe K, Kelly M, Masters M, McCabe J, McIntyre L, Ponce P, Rowland S, Ruiz Ventura J, Swali P, Tait F, Walker D, Webb H, Williams M, Witkin A, Holst M, Loe L, Armit I, Schulting R, Skoglund P
*Commun Biol*. 2024 Jan 11;7(1):14.
doi: 10.1038/s42003-023-05642-z. PMID: 38212558

Brief summary: This study marks significant progress in understanding ancient genomes, particularly in deciphering chromosomal sex, aneuploidies, and their broader historical and societal implications. By extracting and analyzing DNA from ancient remains, researchers have uncovered new insights into disorders of sex development (DSDs) that were previously inaccessible. These authors pioneered a computational method to identify sex chromosomal aneuploidies. This approach independently quantifies the number of observed

sequences aligning to any chromosome and compares them to an 'autosomal baseline,' a sum of sequences aligning to chromosomes 1 through 22, excluding chromosomes 13, 18, and 21, known for their association with autosomal aneuploidies that survive to birth in significant numbers.

This method was applied to 570 ancient genomes from Viking Age Northern Europe and ancient Rome. It identified some of the earliest known cases of sex chromosome syndromes, such as Klinefelter syndrome (KS; 47,XXY), Mosaic Turner syndrome (TS; 45,X/46,XX) and Jacobs syndrome (47,XYY).

The study demonstrates the power of ancient DNA analysis in overcoming the limitations of traditional osteological methods to determine sex, which can be inaccurate, particularly with incomplete skeletal remains or nonadult individuals. The development of computational methods to distinguish between various karyotypes and detect aneuploidies represents a significant advancement in genomic archaeology, enabling more accurate sex identification and insights into the societal context of individuals with aneuploidies in the past.

Identifying aneuploidies like KS, repeatedly observed in ancient genomic records, and detecting other conditions such as TS and Down syndrome offer a nuanced understanding of how these individuals may have been perceived and integrated into their communities. The study suggests that individuals with aneuploidies were generally buried according to the customs of their time, indicating they were considered ordinary members of their communities. Additionally, evidence of delayed growth and the absence of offspring buried nearby may reflect hypogonadism and reduced fertility.

This study advances the technical capabilities of ancient DNA analysis and opens new avenues for exploring the complex relationships between genetics, identity, and society in historical populations. It emphasizes the importance of integrating genomic data with osteological and archaeological evidence to construct a more comprehensive picture of the human past. For a commentary on this article see (1).

Reference

1. Wong C. Ancient DNA reveals first known case of sex-development disorder. *Nature*. 2024 Jan;625(7996):639. doi: 10.1038/d41586-024-00101-z. PMID: 38216764.

## Gender Incongruence - Psychological Benefits of Puberty Suppression

### 6.11. Association of pubertal blockade at Tanner 2/3 with psychosocial benefits in transgender and gender diverse youth at hormone readiness assessment

McGregor K, McKenna JL, Williams CR, Barrera EP, Boskey ER

*J Adolesc Health*. 2024 Apr;74(4):801-807.
doi: 10.1016/j.jadohealth.2023.10.028. PMID: 38099903

Brief summary: This retrospective cohort study compared Youth Self-Report (YSR) scores at their hormone readiness assessment, before the start of gender-affirming sex hormone treatment, between 40 transgender adolescents, who had received puberty suppression from Tanner stage 2-3, and 398 transgender adolescents who had not received puberty suppression. In multivariate analyses adjusting for gender, and using a sensitivity analysis restricted to 13–15-year-olds, the group with puberty suppression showed lower scores for total problems, internalizing problems, depression problems, anxiety problems and stress problems.

Current guidelines recommend puberty suppression with GnRH analogues as an initial treatment for eligible transgender and gender diverse adolescents to prevent the development of unwanted secondary sex characteristics, and thereby improve wellbeing. In many studies of GnRH analogue treatment, only a minority started treatment in early puberty, when irreversible changes such as voice breaking and breast development can still be prevented. The current study included a relatively large number of adolescents who had started GnRH analogue treatment in early puberty (Tanner stage 2 or 3). This treated group had better psychological outcomes compared to adolescents who did not receive puberty suppression.

Although the cross-sectional design does not allow causal inference, and groups may have differed in factors that caused earlier presentation for GnRH analogue treatment in the treated group, the positive impact of GnRH analogue treatment is supported by another recent study. Fisher et al., in a longitudinal study of 36 adolescents, also found improved YSR total, internalizing and depression and anxiety scores after the start of GnRH

analogue treatment (1). These findings are in line with previous findings of less internalizing problems in adolescents treated with GnRH analogue treatment (2, 3), although not confirmed in all studies (4).


References

1. Fisher AD, Ristori J, Romani A, Cassioli E, Mazzoli F, Cocchetti C, Pierdominici M, Marconi M, Ricca V, Maggi M, Vignozzi L, Castellini G. Back to the Future: Is GnRHa Treatment in Transgender and Gender Diverse Adolescents Only an Extended Evaluation Phase? *J Clin Endocrinol Metab.* 2024 May 17;109(6):1565-1579. doi: 10.1210/clinem/dgad729. PMID: 38099569.
2. van der Miesen AIR, Steensma TD, de Vries ALC, Bos H, Popma A. Psychological Functioning in Transgender Adolescents Before and After Gender-Affirmative Care Compared With Cisgender General Population Peers. *J Adolesc Health.* 2020 Jun;66(6):699-704. doi: 10.1016/j.jadohealth.2019.12.018. Epub 2020 Apr 6. PMID: 32273193.
3. de Vries AL, Steensma TD, Doreleijers TA, Cohen-Kettenis PT. Puberty suppression in adolescents with gender identity disorder: a prospective follow-up study. *J Sex Med.* 2011 Aug;8(8):2276-83. doi: 10.1111/j.1743-6109.2010.01943.x. Epub 2010 Jul 14. PMID: 20646177.
4. Carmichael P, Butler G, Masic U, Cole TJ, De Stavola BL, Davidson S, et al. Short-term outcomes of pubertal suppression in a selected cohort of 12 to 15 year old young people with persistent gender dysphoria in the UK. *PLoS One.* 2021;16(2):e0243894


## Gender Incongruence - Hormone Treatment Continuation

## 6.12. Reidentification with birth-registered sex in a Western Australian pediatric gender clinic cohort


Cavve BS, Bickendorf X, Ball J, Saunders LA, Thomas CS, Strauss P, Chaplyn G, Marion L, Siafarikas A, Ganti U, Wiggins A, Lin A, Moore JK



*JAMA Pediatr.* 2024 May 1;178(5):446-453.
doi: 10.1001/jamapediatrics.2024.0077. PMID: 38436975


Brief summary: This retrospective cohort study investigated referral closures due to reidentification with birth-registered sex at the Child and Adolescent Health Service Gender Diversity Service in Perth, the sole provider of gender-affirming medical treatment for individuals <18 years in Western Australia. Out of 548 referral closures, 29 reidentified with birth-registered sex. Only 2 did so after having started medical treatment, constituting 1.2% (95% CI, 0.1%-4.4%) of all adolescents who had initiated puberty suppression and 0.8% (95% CI, 0.0%-4.1%) of those who had initiated gender-affirming sex hormone treatment.

A few studies, from the US and Europe, previously investigated treatment continuation in transgender and gender diverse adolescents. They had reported continuation rates for gender affirming sex hormone treatment varying from 74% after 4 years to 98% after a median 3.5 years (IQR 1.5–7.6; range 0.1–20·0) for transfeminine and 2.3 years (1.2–4.8; range 0.0–15.5) for transmasculine individuals (1-3). This study from Australia, which has seen a similar exponential increase in referrals of adolescents for gender affirming care as Europe and the US, found that of adolescents who reidentified with their birth-registered sex, nearly all did so before they started medical treatment. Another recent retrospective cohort study by Gupta et al. from Atlanta, US, found that out of 121 adolescents who started gender affirming sex hormone treatment before age 18 years, only 3 temporarily discontinued treatment and only 1 permanently stopped, but none reidentified with their birth-registered gender and none expressed regret about receiving the treatment (4). Reasons for (temporarily) stopping treatment were bullying, insurance issues, wanting to conceive a baby and transition to a non-binary gender.

Discontinuation of treatment seems uncommon in the Australian and European studies, can have many reasons and is not necessarily associated with retransition or regret. Further research is necessary to better understand retransition and optimise care for those who wish to retransition.


References

1. van der Loos MATC, Hannema SE, Klink DT, den Heijer M, Wiepjes CM. Continuation of gender-affirming hormones in transgender people starting puberty suppression in adolescence: a cohort study in the Netherlands. *Lancet Child Adolesc Health.* 2022 Dec;6(12):869-875. doi: 10.1016/S2352-4642(22)00254-1. Epub 2022 Oct 21. PMID: 36273487.
2. Butler G, Adu-Gyamfi K, Clarkson K, El Khairi R, Kleczewski S, Roberts A, Segal TY, Yogamanoharan K, Alvi S, Amin N, Carruthers P, Dover S, Eastman J, Mushtaq T, Masic U, Carmichael P. Discharge outcome analysis of 1089 transgender young people referred to paediatric endocrine clinics in England 2008–2021. *Arch Dis Child.* 2022 Jul 18:archdischild-2022-324302. doi:*10.1136/archdischild-2022-324302*. Epub ahead of print. PMID: 35851291.

3. Roberts CM, Klein DA, Adirim TA, Schvey NA, Hisle-Gorman E. Continuation of Gender-affirming Hormones Among Transgender Adolescents and Adults. *J Clin Endocrinol Metab.* 2022 Aug 18;107(9):e3937–e3943. doi: 10.1210/clinem/dgac251. PMID: 35452119.
4. Gupta P, Patterson BC, Chu L, Gold S, Amos S, Yeung H, Goodman M, Tangpricha V. Adherence to Gender Affirming Hormone Therapy in Transgender Adolescents and Adults: A Retrospective Cohort Study. *J Clin Endocrinol Metab.* 2023 Oct 18;108(11):e1236-e1244. doi: 10.1210/clinem/dgad306. PMID: 37246711; PMCID: PMC10583985.

## Impact of Masculinizing Hormone Treatment on Fertility

### 6.13. One-third of amenorrheic transmasculine people on testosterone ovulate

Asseler JD, Del Valle JS, Chuva de Sousa Lopes SM, Verhoeven MO, Goddijn M, Huirne JAF, van Mello NM

*Cell Rep Med*. 2024 Mar 19;5(3):101440.

doi: 10.1016/j.xcrm.2024.101440. PMID: 38402622

Brief summary: This study analyzed ovariectomy samples from 52 transmasculine individuals who had been using testosterone for > 1 year (median 32, IQR 27–39 months) with serum concentrations in the male reference range. In one third (16/52) of the cohort, histological signs of recent ovulatory activity were observed, including the presence of ovulatory follicles, corpus luteum and corpus albicans. This was similar in the subgroup of 15 individuals who had previously used a GnRH analogue, discontinued at least 7 months prior to ovariectomy.

Testosterone treatment generally induces amenorrhea in transmasculine individuals. However, this study shows that ovulation may nonetheless occur. This underlines the importance of informing transgender individuals on testosterone therapy that there may be a chance of pregnancy despite amenorrhea and the need for contraception if they engage in sexual activity that can result in pregnancy. The findings are in line with that of a recent study using a mouse model of gender affirming treatment for adolescents (1). Mice were treated with leuprolide acetate and/or testosterone. 2/8 mice treated with testosterone showed evidence of cycling and a high number of corpora lutea on histology. That study also showed that eggs from mice treated with leuprolide acetate to prevent puberty and subsequently with testosterone, could be fertilized resulting in similar rates of live births to controls, and in fertile offspring (1). In humans, successful oocyte cryopreservation, fertilization and live birth as well as natural conception have also been reported in transmasculine persons after testosterone treatment albeit in small cohorts (2-4).

Together, these findings are reassuring with regard to the impact of gender-affirming hormone treatment on fertility in transmasculine individuals.

References

1. Godiwala P, Uliasz TF, Lowther KM, Kaback D, Mehlmann LM. Puberty Suppression Followed by Testosterone Therapy Does Not Impair Reproductive Potential in Female Mice. *Endocrinology.* 2023 Sep 23;164(11):bqad145. doi: 10.1210/endocr/bqad145. PMID: 37768169.
2. Leung A, Sakkas D, Pang S, Thornton K, Resetkova N. Assisted reproductive technology outcomes in female-to-male transgender patients compared with cisgender patients: a new frontier in reproductive medicine. *Fertil Steril*. 2019 Nov;112(5):858-865. doi: 10.1016/j.fertnstert.2019.07.014. Epub 2019 Oct 6. PMID: 31594633.
3. Light AD, Obedin-Maliver J, Sevelius JM, Kerns JL. Transgender men who experienced pregnancy after female-to-male gender transitioning. *Obstet Gynecol.* 2014 Dec;124(6):1120-1127. doi: 10.1097/AOG.0000000000000540. PMID: 25415163.
4. Asseler JD, Knieriem J, Huirne JA, Goddijn M, Verhoeven MO, van Mello NM. Outcomes of oocyte vitrification in trans masculine individuals. *Reprod Biomed Online*. 2023 Jul;47(1):94-101. doi: 10.1016/j.rbmo.2023.03.007. Epub 2023 Mar 14. PMID: 37095040.

## Bone Health after Puberty Suppression and Gender Affirming Hormone Treatment

### 6.14. Bone mineral density in transgender adolescents treated with puberty suppression and subsequent gender-affirming hormones

van der Loos MATC, Vlot MC, Klink DT, Hannema SE, den Heijer M, Wiepjes CM

*JAMA Pediatr.* 2023 Dec 1;177(12):1332-1341.

doi: 10.1001/jamapediatrics.2023.4588. PMID: 37902760

Brief summary: This prospective follow-up cohort study investigated bone mineral density in transgender individuals who had initiated GnRH analogue treatment before age 18 followed by sex hormone treatment for at least 9 years. At a median age 28.2 years, after using testosterone for a median 11.9 years (IQR 10.2-13.8), transmasculine individuals had mean z-scores close to 0 at the lumbar spine, total hip and femoral neck, similar to z-scores at the start of GnRH analogue treatment. In contrast, transfeminine individuals at a median age 28.2 years, after using estradiol for a median 11.6 years (IQR 10.1-14.7) had mean z-scores of −1.34 at the lumbar spine, −0.66 at the total hip, and −0.54 at the femoral neck. Only at the lumbar spine was the z-score lower than that at the start of GnRH analogue treatment (difference −0.87; 95% CI, −1.15 to −0.59).

Concerns exist about the potential long-term impact of puberty suppression with GnRH analogues on bone health. GnRH analogue treatment is known to reduce bone mineral accrual in transgender and gender diverse adolescents, but this study shows that complete catch-up is seen with long-term testosterone treatment. With estradiol treatment, z-scores at the total hip and femoral neck also returned to (below average) pre-treatment values, but not at the lumbar spine. The authors suggest this may be due to insufficient estradiol concentrations. Indeed, a higher estrogen dosage has been found to be associated with a greater increase in lumbar spine BMD Z-scores (1). Since z-scores were already below average before the start of any treatment, lifestyle factors likely also play a role in suboptimal bone health in transfeminine individuals and deserve attention during clinical follow-up.

Animal models are now also being used to assess the impact of hormonal interventions on bone and allow additional aspect of bone health such as bone architecture to be studied. Dubois et al., using a mouse model of puberty suppression followed by testosterone treatment, found that GnRH analogue treatment results in lower trabecular bone volume, cortical bone mass and cortical strength and higher bone marrow adiposity, all of which were restored by testosterone (2). Thus, in transmasculine individuals, GnRH analogue treatment followed by testosterone treatment seems safe with regard to bone health. However, bone health in transfeminine individuals deserves attention, with counseling about the importance of weight-bearing exercise, sufficient calcium intake and vitamin D.

References

1. Boogers LS, van der Loos MATC, Wiepjes CM, van Trotsenburg ASP, den Heijer M, Hannema SE. The dose-dependent effect of estrogen on bone mineral density in trans girls. *Eur J Endocrinol.* 2023 Aug 2;189(2):290-296. doi: 10.1093/ejendo/lvad116. PMID: 37590955.
2. Dubois V, Ciancia S, Doms S, El Kharraz S, Sommers V, Kim NR, David K, Van Dijck J, Valle-Tenney R, Maes C, Antonio L, Decallonne B, Carmeliet G, Claessens F, Cools M, Vanderschueren D. Testosterone Restores Body Composition, Bone Mass, and Bone Strength Following Early Puberty Suppression in a Mouse Model Mimicking the Clinical Strategy in Trans Boys. *J Bone Miner Res.* 2023 Oct;38(10):1497-1508. doi: 10.1002/jbmr.4832. Epub 2023 Jun 8. PMID: 37222072.

## Cardiovascular Outcomes in Transgender People

### 6.15. Cardiovascular disease in transgender people: a systematic review and meta-analysis

van Zijverden LM, Wiepjes CM, van Diemen JJK, Thijs A, den Heijer M

*Eur J Endocrinol*. 2024 Feb 1;190(2):S13-S24.
doi: 10.1093/ejendo/lvad170. PMID: 38302717

Brief summary: This systematic review and meta-analysis included 22 studies, with a total of 19,893 transgender women, 14,840 transgender men, 371,547 cisgender men, and 434,700 cisgender women, to assess the occurrence of cardiovascular events. The risk of the combined outcome (stroke, myocardial infarction or thrombosis) was 40% higher in transgender people compared to cisgender people of the same birth-registered sex.

Gender-affirming therapy may play role in the increased risk of cardiovascular morbidity and mortality. Estrogen therapies are associated with increased risk of venous thromboembolism; transgender women were indeed found to have a relative risk of 2.2 (95% CI, 1.1-4.5) for thrombosis compared to cisgender men. Gender-affirming surgeries, like any major surgeries, may also predispose to thromboembolism. On the other hand, the risk of arterial cardiovascular events was similar in transgender men compared to transgender women, which seems

surprising as testosterone therapy (but not estrogen) has been associated with an adverse impact on cardiovascular risk factors and with a higher risk of subclinical atherosclerosis in transgender individuals (1,2). This suggests that non-hormonal factors may play a role, such as minority stress, socioeconomic conditions and lifestyle factors.

Prospective studies that collect information on these factors are necessary to better understand the underlying causes of the increased cardiovascular morbidity in transgender people, and to compare the risk between different hormone formulations, doses and routes of administration. In the meantime, cardiovascular risk management should be an integral part of transgender care.

References


1. Gosiker B, Moutchia J, Nguyen N, Getahun D, Goodman M. Changes in Blood Lipids Following Initiation of Gender Affirming Hormone Therapy: A Systematic Review and Meta-Analysis. *J Clin Transl Endocrinol.* 2024 Apr 30;36:100349. doi: 10.1016/j.jcte.2024.100349. PMID: 38737626; PMCID: PMC11087959.
2. Moreira Allgayer RMC, Borba GDS, Moraes RS, Ramos RB, Spritzer PM. The Effect of Gender-Affirming Hormone Therapy on the Risk of Subclinical Atherosclerosis in the Transgender Population: A Systematic Review. *Endocr Pract.* 2023 Jun;29(6):498-507. doi: 10.1016/j.eprac.2022.12.017. Epub 2023 Jan 2. PMID: 36603652.

# 7. Puberty

Julie Fudvoye[1,2], Caroline Gernay[1], Anne-Simone Parent[1,2]
[1]Department of Pediatrics, University Hospital Liège, Belgium; [2]Neuroendocrinology Unit, GIGA-Neurosciences, University of Liège, Belgium

## Introduction

This year clinical studies explored further some persistent questions regarding the challenges of diagnosing precocious or delayed puberty. Studies also explored further the genetic architecture of pathological puberty and identified rare new variants explaining central precocious puberty as well as common variants participating to constitutional delay of growth and puberty. The studies summarized in this chapter underline the future importance of polygenic scores for exploring abnormal puberty.

Basic science research this year illustrates the importance of the regulation of reproductive maturation by energy accessibility. Glutamate transmission in leptin sensitive neurons is required for normal pubertal development and bile acids appear to define a new role for the hepatic-gut-neuroendocrine axis in the control of puberty.

## Clinical Guidance and Studies

### 7.1. Girls with idiopathic central precocious puberty did not display substantial changes in body mass index after treatment with gonadotropin-releasing hormone analogues

Uldbjerg CS, Lim YH, Renault CH, Hansen D, Juul A, Bräuner EV, Jensen RB
*Acta Paediatr.* 2024 Jul;113(7):1602-1611.
doi: 10.1111/apa.17185. PMID: 38506052.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/apa.17185

Brief summary: This retrospective study of 123 Danish girls treated with GnRHa for idiopathic central precocious puberty (CPP) shows that GnRH agonists do not impact body mass index during and after cessation of treatment.

The use of GnRH agonists (GnRHa) for treatment of girls with CPP is well established but the potential long term effects of GnRHa remain a matter of debate, especially regarding their impact on weight evolution[1,2].

The aim of this study was to evaluate the effects of GnRHa on body mass index (BMI) trajectories during treatment (median 18.9 months) and after cessation of treatment (median 18.2 months). The authors postulated that effects of GnRHa treatment on BMI were correlated with baseline BMI (measured within +/- 1 month of initiation for 87% of girls) and with GnRHa treatment protocol (leuprolide acetate treatment: 4 or 3 weekly intervals with 3.75 mg or 12 or 9 weekly intervals with 11.25 mg or mixed protocol).

No overall changes in BMI SDS were observed during and after GnRHa treatment period. Girls in the lowest baseline BMI group (n = 22) had a weak trend towards an ascending change in BMI SDS during treatment, while there was no clear pattern for BMI SDS changes after treatment. Overall, BMI SDS trajectories during treatment with GnRHa or after cessation of treatment were not impacted by the three dosage-interval protocols. Those findings suggest that GnRHa treatment does not significantly impact BMI in girls with idiopathic CPP.

Those results are in line with previous studies overall11However, others have reported a worsening of metabolic parameters such as insulin sensitivity under GnRHa treatment[5]. In the current study, the lack of a control group to compare treated and untreated girls with CPP is a limitation, highlighting the need for further large cohort studies to evaluate the long-term effects of GnRHa treatment on BMI and metabolic profiles.

References

1. Corripio R, Soriano-Guillén L, Herrero FJ, et al. Changes in body mass index in girls with idiopathic central precocious puberty under gonadotropin-releasing hormone analogue therapy: the Spanish registry. *Horm Res Paediatr.* 2016;86(3):154-60.
2. Luo X, Liang Y, Hou L, Wu W, Ying Y, Ye F. Long-term efficacy and safety of gonadotropin-releasing hormone analog treatment inchildren with idiopathic central precocious puberty: a systematic review and meta-analysis. *Clin Endocrinol.* 2021;94(5):786-96.
3. Liang Y, Wei H, Li J, et al. Effect of GnRHa 3.75 mg subcutaneously every 6 weeks on adult height in girls with idiopathic central precocious puberty. *J Pediatr Endocrinol Metab.* 2015;28(7–8):839-46.
4. Poomthavorn P, Suphasit R, Mahachoklertwattana P. Adult height, body mass index and time of menarche of girls with idiopathic central precocious puberty after gonadotropin- releasing hormone analogue treatment. *Gynecol Endocrinol.* 2011;27(8):524-8.

## 7.2. Differentiation of idiopathic central precocious puberty from premature thelarche using principal component analysis

Cleemann Wang A, Hagen CP, Johannsen TH, Madsen AG, Cleemann LH, Christiansen P, Main KM, Juul A, Jensen RB
*J Clin Endocrinol Metab*. 2024 Jan 18;109(2):370-379.
doi: 10.1210/clinem/dgad535. PMID: 37698163.
https://pubmed.ncbi.nlm.nih.gov/37698163/

Brief summary: this retrospective study describes clinical and biochemical parameters which, using the principal component analysis, help in the differential diagnosis between idiopathic central precocious puberty (ICPP) and premature thelarche (PT).

Breast development in girls before 8 years of age may be related to progressive CPP but can sometimes simply be the consequence of premature thelarche without activation of the hypothalamic-pituitary-gonadal axis.

This study showed that clinical, anthropometric, radiological, and biochemical markers in combination can distinguish between these two entities without need for GnRH stimulation testing

Between 2009 to 2019, 1316 girls were referred to a single tertiary centre of pediatric endocrinology in Denmark and included in the study. After applying exclusion criteria, 474 patients remained and were divided according to diagnosis (idiopathic central precocious puberty, organic central precocious puberty, premature thelarche, premature adrenarche, peripheral precocious puberty). ICPP in girls was defined as Tanner stage $\geq$ B2 before 8 years of age, and either a basal concentration of LH $>$0.3 IU/L and/or a pubertal response to a GnRH stimulation test (peak LH $>$ 5 IU/L)[1].

Receiver operating analyses allowed the identification of markers to differentiate between girls with ICPP and PT (advancement in bone age, serum concentrations of basal FSH, basal LH, testosterone, androstenedione, and IGF-I). The use of principal component analysis increased their diagnostic value, which remains low when using them individually. Principal component analysis-derived clinical and hormone profiles could predict girls with ICPP from girls with PT with a specificity of 90% and sensitivity of 84%, outperforming any single marker. This offers clinicians an alternative approach in the early outpatient setting and before resorting to a stimulation test.

Reference

1. Neely EK, Wilson DM, Lee PA, Stene M, Hintz RL. Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. *J Pediatr.* 1995;127(1):47-52.

## 7.3. Sex-independent timing of the onset of central puberty revealed by nocturnal luteinizing hormone concentrations

Demir A, Hero M, Juul A, Main KM
*Clin Endocrinol (Oxf)*. 2023 Dec;99(6):552-558.
doi: 10.1111/cen.14974. PMID: 37772429.
https://onlinelibrary.wiley.com/share/I5IKBQ75GYGBI98BG63X?target=10.1111/cen.14974

Brief summary: this longitudinal study found no statistical difference between boys and girls regarding the timing of increase in nocturnal luteinizing hormone concentrations.

Puberty results from a gradual increase in GnRH secretion, that can be monitored by urinary gonadotropin measurements. Timing of normal puberty, initially described by Marshal and Tanner, is characterized by an important individual variability in humans[1,2]. The difference in puberty timing between boys and girls as well as the higher incidence of reported precocious puberty in girls[3,4,] compared to boys and delayed puberty in boys[5,6] compared to girls remain unexplained.

The aim of this study was to investigate the temporal association between activation of the hypothalamic-pituitary-gonadal axis, evaluated by urinary LH, and clinical signs of puberty onset in both sexes. Thirty subjects were included. They were examined by a single observer every 3 to 4 months during 5.5 and 5.8 years on average. Participants provided 24h-urine sample divided into nocturnal sleep and waketime at each visit for determination of LH concentration (U-LH). The cutoff level used for the determination of the onset of puberty was determined at 0.7 U/L.

The initial increase of U-LH concentrations was detectable only in nocturnal sleeptime samples and occurred at around the same age (9-10 years of age) in both sexes. The time between this gonadotropin increase and the appearance of the first clinical signs of puberty was significantly longer in boys (1.5 years) than in girls (0.1 years).

In conclusion, this study showed a sex-independent increase in urinary LH at age 9-10 in a small cohort, with a longer time span to clinical puberty in boys. This suggests that testes could require longer gonadotropin stimulation before reaching tanner stage 2 compared to breasts. However, one cannot exclude that breast development observed in some girls might initially be isolated thelarche or adipomastia.

References


1. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44(235): 291-303.
2. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in boys. *Arch Dis Child.* 1970;45(239): 13-23.
3. Bridges NA, Christopher JA, Hindmarsh PC, Brook CG. Sexual precocity: sex incidence and aetiology. *Arch Dis Child.* 1994;70(2):116-118.
4. Chemaitilly W, Trivin C, Adan L, Gall V, Sainte-Rose C, Brauner R.Central precocious puberty: clinical and laboratory features. *ClinEndocrinol.* 2001;54(3):289-294.
5. Bourguignon JP. Growth and timing of puberty: reciprocal effects. *Horm Res.* 1991;36(3-4):131-135.
6. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large caseseries from an academic center. *J Clin Endocrinol Metab.* 2002;87(4):1613-1620.


## 7.4. Unstimulated luteinizing hormone for assessment of suppression during treatment of central precocious puberty with 6-month subcutaneous leuprolide acetate: correlations with clinical response


Klein KO, Miller BS, Mauras N

*Horm Res Paediatr.* 2024 Apr 29:1-10.

doi: 10.1159/000539110. PMID: 38684152.

https://karger.com/hrp/article/doi/10.1159/000539110/906770/Unstimulated-Luteinizing-Hormone-for-Assessment-of


Brief summary: This prospective study of 62 children with Central Precocious Puberty (CPP) concluded that measuring unstimulated luteinizing hormone (LH) may be adequate to assess effective pubertal suppression.

Pubertal stages, growth velocity and bone age maturation are considered as key clinical markers to evaluate adequate pubertal suppression in patients treated for CPP. Basal LH concentrations levels also seem to be effective[1]. This study validated unstimulated LH level for assessing pubertal suppression in children with CPP.

Data were collected in patients enrolled in a phase 3 trial to assess efficacy and safety of a small-volume subcutaneous 6-Month Duration Leuprolide Acetate (LA)[2].

Unstimulated LH levels were $<$ 1 IU/L in 84% and 86% of children at weeks 24 and 48 respectively. Eight children did not achieve unstimulated LH $<$ 1 IU/L at week 24 but stopped pubertal stage progression and had stable or decreased bone age to chronological age ratio. Seven girls did not achieve stimulated LH $<$5 IU/L but

none of them showed any progression in pubertal staging. A positive correlation was found between GnRH-stimulated and unstimulated LH at week 24 and 48 (($r2 = 0.508$ and 0.550 respectively). No correlation was found between unstimulated LH and clinical endpoints.

This study reveals that unstimulated LH $< 1$ UI/L may serve as a marker of pubertal suppression in children with CPP treated with 6-month subcutaneous LA. However, the absence of correlation between LH concentrations and clinical growth endpoints highlights the need to integrate all clinical parameters with any biological data.

References

1. Peter A Lee, Margaret Luce, Peter Bacher J. Monitoring treatment of central precocious puberty using basal luteinizing hormone levels and practical considerations for dosing with a 3-month leuprolide acetate formulation. *Pediatr Endocrinol Metab.* 2016; 29(11):1249-1257.
2. Klein KO, Freire A, Gryngarten MG, Kletter GB, Benson M, Miller BS, Dajani TS, Eugster EA, Mauras N. Phase 3 Trial of a Small-volume Subcutaneous 6-Month Duration Leuprolide Acetate Treatment for Central Precocious Puberty. *J Clin Endocrinol Metab.* 2020;105(10):e3660-71.

## 7.5. Rare variants in the MECP2 gene in girls with central precocious puberty: a translational cohort study

Canton APM, Tinano FR, Guasti L, Montenegro LR, Ryan F, Shears D, de Melo ME, Gomes LG, Piana MP, Brauner R, Espino-Aguilar R, Escribano-Muñoz A, Paganoni A, Read JE, Korbonits M, Seraphim CE, Costa SS, Krepischi AC, Jorge AAL, David A, Kaisinger LR, Ong KK, Perry JRB, Abreu AP, Kaiser UB, Argente J, Mendonca BB, Brito VN, Howard SR, Latronico AC

*Lancet Diabetes Endocrinol*. 2023 Aug;11(8):545-554.

doi: 10.1016/S2213-8587(23)00131-6. PMID: 37385287.

https://www.sciencedirect.com/science/article/pii/S2213858723001316?via%3Dihub

Brief summary: This international cohort study of 404 patients identified rare likely damaging variants in the gene *MECP2* in patients with central precocious puberty. Translational experiments showed that GnRH neurons in mice express *Mecp2*.

Over the last few years, several studies have provided insight into the epigenetic regulation of the onset of puberty[1-3]. DNA methylation, histone post-translational modifications and non-coding RNAs have a crucial role in the regulation of the transcriptional machinery of neurons involved in reactivating the GnRH pulse generator around puberty. Identification of genetic causes of central precocious puberty has confirmed the role of epigenetic mechanisms in the control of puberty[4,5]. This study provides additional insights into the function of chromatin organisation in GnRH neurons.

*MECP2* is an X-linked gene encoding for a chromatin-associated protein. Loss of function mutations of *MECP2* usually cause Rett syndrome, which is a severe neurodevelopmental disorder associated with early onset of puberty.

Between 2020 and 2022, 404 patients with sporadic or familial idiopathic central precocious puberty (95% girls and 5% boys) were included in this genetic study. Whole-exome sequencing was used for 62 patients and targeted gene sequencing for 71 patients. Additionally, 271 patients with isolated central precocious puberty were screened for *MECP2* by Sanger sequencing.

Three rare heterozygous likely damaging coding variants in *MECP2* were identified in five girls. These patients presented microcephaly and/or neurocognitive phenotype but criteria for Rett syndrome were not met. Additionally, one rare heterozygous 3′UTR *MECP2* insertion was identified in two unrelated girls with sporadic central precocious puberty. None of them manifested Rett syndrome. Three of the four *MECP2* variants were located in the coding region and were predicted to be damaging by in-silico approaches. *MECP2* variants were classified as likely pathogenic in 3 of the 7 girls and variant of unknown significance in 4 girls using ACMG criteria. Analysis of the UK Biobank data did not identify an association between precocious menarche and rare protein variants in *MECP2*, although data on age at thelarche and pubarche were not available. Authors showed that Mecp2 is expressed in the hypothalamus including the arcuate, suprachiasmatic, and paraventricular nuclei, and in the median eminence of pubertal female mice. Co-immunostaining showed that 70% of GnRH neurons expressed Mecp2.

In summary, this study suggest a potential X-linked form of central precocious puberty associated with rare variants in *MECP2*, a key component of human DNA methylation regulation expressed in GnRH neurons.


References

1. Lomniczi, A., Aylwin, C., Vigh-Conrad, K. The Emerging Role of Chromatin Remodeling Factors in Female Pubertal Development. *Neuroendocrinology* 2019; 109(3):208-217.
2. Toro, C. A., Wright, H., Aylwin, C. F., Ojeda, S. R. & Lomniczi, A. Trithorax dependent changes in chromatin landscape at enhancer and promoter regions drive female puberty. *Nat. Commun.* 2018; 9: 57.
3. Tomikawa J, Uenoyama Y, Ozawa M, Fukanuma T, Takase K, Goto T, Abe H, Ieda N, Minabe S, Deura C, Inoue N, Sanbo M, Tomita K, Hirabayashi M, Tanaka S, Imamura T, Okamura H, Maeda K, Tsukamura H. Epigenetic regulation of Kiss1 gene expression mediating estrogen-positive feedback action in the mouse brain. *Proc Natl Acad Sci U S A.* 2012;109(20):E1294-301.
4. Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB. Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med.* 2013;368(26):2467-75.
5. Dauber A, Cunha-Silva M, Macedo DB, Brito VN, Abreu AP, Roberts SA, Montenegro LR, Andrew M, Kirby A, Weirauch MT, Labilloy G, Bessa DS, Carroll RS, Jacobs DC, Chappell PE, Mendonca BB, Haig D, Kaiser UB, Latronico AC. Paternally Inherited DLK1 Deletion Associated With Familial Central Precocious Puberty. *J Clin Endocrinol Metab.* 2017;102(5):1557-1567.


## 7.6. Familial central precocious puberty due to DLK1 deficiency: novel genetic findings and relevance of serum DLK1 levels


Montenegro L, Seraphim C, Tinano F, Piovesan M, Canton APM, McElreavey K, Brabant S, Boris NP, Magnuson M, Carroll RS, Kaiser UB, Argente J, Barrios V, Brito VN, Brauner R, Latronico AC



*Eur J Endocrinol*. 2023 Sep 1;189(3):422-428.

doi: 10.1093/ejendo/lvad129. PMID:37703313.

https://academic.oup.com/ejendo/article/189/3/422/7273070


Brief summary: This cross-sectional study identifies two pathogenic variants in the Delta-like noncanonical notch ligand 1 (*DLK1*) gene in a French cohort of 121 children with idiopathic central precocious puberty (CPP).

DLK1 is a noncanonical ligand of the Delta Notch pathway known to be involved in adipocyte differentiation. Its hypothalamic expression suggests a potential role in coordinating reproductive and metabolic functions. Pathogenic variants in the *DLK1* gene have been found in children presenting CPP[1,2]. In this article, the authors hypothesized that measurement of the soluble form of DLK1 could offer a potential screening tool for patients with CPP.

121 French individuals with CPP (98 girls and 23 boys) had DNA sequenced for *DLK1*. Additionally, mean DLK1 serum levels were measured in 209 individuals (115 girls and 94 boys) from Brazilian and Spanish cohorts (18 with CPP and 191 with normal pubertal development). Translational experiments evaluated DLK1 levels at different stages of pubertal maturation in 5 female mice.

Two novel loss of function mutations in *DLK1* were identified in two girls with non-syndromic CPP, inherited from their unaffected carrier father. Mean DLK1 levels were not different between patients with CPP and normal puberty ($7.9 \pm 3.6$ and $8.2 \pm 3$ ng/mL, $P = 0.79$). In children with normal pubertal development, serum DLK1 levels decreased across pubertal development with a sex specific pattern: in girls ($n = 97$), DLK1 levels decreased from Tanner III to Tanner V while in boys ($n = 94$), DLK1 levels decreased from Tanner II to V. No association was found between DLK1 levels and BMI SDS after linear regression analysis. In mice, serum DLK1 levels decreased gradually during pubertal maturation.

In summary, 2 novel mutations were described in *DLK1* gene in two girls with CPP. In addition, the changes in DLK1 serum levels during puberty suggests a role for this factor in regulating pubertal development but do not help to distinguish between CPP and normal puberty.


References

1. Andrew Dauber, Marina Cunha-Silva, Delanie B. Macedo, Vinicius N. Brito, Ana Paula Abreu, Stephanie A. Roberts, Luciana R. Montenegro, Melissa Andrew, Andrew Kirby, Matthew T. Weirauch, Guillaume Labilloy, Danielle S. Bessa, Rona S. Carroll, Dakota C. Jacobs, Patrick E. Chappell, Berenice B. Mendonca, David Haig, Ursula B. Kaiser, and Ana Claudia Latronico. Paternally Inherited *DLK1* Deletion Associated With Familial Central Precocious Puberty. *J Clin Endocrinol Metab.* 2017; 102(5): 1557–1567.
2. Novel Genetic and Biochemical Findings of DLK1 in Children with Central Precocious Puberty: A Brazilian-Spanish Study. Luciana Montenegro, José I Labarta, Maira Piovesan, Ana P M Canton, Raquel Corripio, Leandro Soriano-Guillén, Lourdes Travieso-Suárez, Álvaro Martín-Rivada, Vicente Barrios, Carlos E Seraphim, Vinicius N Brito, Ana Claudia Latronico, Jesús Argente Novel Genetic and Biochemical Findings of DLK1 in Children with Central Precocious Puberty: A Brazilian-Spanish Study. *J Clin Endocrinol Metab.* 2020; 105(10):dgaa461.

## 7.7. Contributions of common genetic variants to constitutional delay of puberty and idiopathic hypogonadotropic hypogonadism

Lippincott MF, Schafer EC, Hindman AA, He W, Brauner R, Delaney A, Grinspon R, Hall JE, Hirschhorn JN, McElreavey K, Palmert MR, Rey R, Seminara SB, Salem RM, Chan YM, Delayed Puberty Genetics Consortium

*J Clin Endocrinol Metab*. 2024 Mar 13:dgae166.
doi: 10.1210/clinem/dgae166. PMID: 38477512.
https://pubmed.ncbi.nlm.nih.gov/38477512/

Brief summary: this case-control study shows that the common genetic variants that influence pubertal timing in the general population also contribute to constitutional delay of puberty (CDP) and less significantly to normosmic idiopathic hypogonadotropic hypogonadism (IHH).

CDP and IHH are two different conditions that are notoriously difficult to distinguish clinically on initial presentation. Because CDP has clear heritability traits[1], and half of individuals with IHH have an identified genetic cause[2], the authors hypothesized that common genetic variants could help to separate these conditions.

Polygenic scores (PGS) were established for pubertal timing (age at voice-breaking and facial hair for boys and age at menarche for girls) based on genome-wide association studies[3,4]. 80 individuals with CDP, 301 with normosmic IHH and 348 with Kallmann syndrome (KS) were analysed. DNA was extracted from saliva and blood samples. PGS scores were established and then compared to controls (9222 genotyped on the same platform as the CDP cohort, and 1868 on the same platform as the normosmic IHH and KS cohort).

There was a strong contribution of common genetic variants to CDP, and only a small contribution to IHH, supporting the idea that they are distinct entities that share some pathophysiological pathways. By contrast, the common genetic variants did not appear to contribute to Kallmann syndrome.

References

1. Sedlmeyer IL, Hirschhorn JN, Palmert MR. Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab.* 2002;87(12):5581-5586.
2. Al Sayed Y, Howard SR. Panel testing for the molecular genetic diagnosis of congenital hypogonadotropic hypogonadism—a clinical perspective. *Eur J Hum Genet.* 2023;31(4):387-394.
3. Day FR, Thompson DJ, Helgason H, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet.* 2017;49(6):834-841.
4. Hollis B, Day FR, Busch AS, et al. Genomic analysis of male puberty timing highlights shared genetic basis with hair colour and lifespan. *Nat Commun.* 2020;11(1):1536.

## 7.8. Prevalence of deleterious variants in *MC3R* in patients with constitutional delay of growth and puberty

Duckett K, Williamson A, Kincaid JWR, Rainbow K, Corbin LJ, Martin HC, Eberhardt RY, Huang QQ, Hurles ME, He W, Brauner R, Delaney A, Dunkel L, Grinspon RP, Hall JE, Hirschhorn JN, Howard SR, Latronico AC, Jorge AAL, McElreavey K, Mericq V, Merino PM, Palmert MR, Plummer L, Rey RA, Rezende RC, Seminara SB, Salnikov K, Banerjee I, Lam BYH, Perry JRB, Timpson NJ, Clayton P, Chan YM, Ong KK, O'Rahilly S

*J Clin Endocrinol Metab*. 2023;108(12):e1580-e1587.
doi: 0.1210/clinem/dgad373. PMID: 37339320.
https://academic.oup.com/jcem/article/108/12/e1580/7204094?login=true

Brief summary: this large patient cohort study identified an overrepresentation of functionally damaging variants in *MC3R* in individuals with constitutional delay of growth and puberty but not in patients with IHH.

Melanocortin 3 receptor (MC3R) is a permissive signal expressed by hypothalamic kisspeptin-neurokinin B-dynorphin (KNDY) neurons. It activates puberty through the leptin-proopiomelanocortin pathway in response to nutritional signaling[1]. Variants in *MC3R* have been associated with later age at puberty and a severely disruptive variant has been recently reported to be associated with extreme pubertal delay[1]. The authors hypothesized that deleterious variants of *MC3R* could also be associated with constitutional delay of growth and puberty (CDGP).

*MC3R* was sequenced in 362 adolescents with CDGP and 657 patients with normosmic idiopathic hypogonadotropic hypogonadism (nIHH). Variant frequencies in those groups were compared to 5774 controls from a population-based cohort. Loss-of-function variants were infrequent but overrepresented in patients with CDGP compared to controls (OR =4.17; $P$=.001). There was no evidence of overrepresentation in patients with nIHH (OR =1.15; $P$=.779). In 246,328 women from the UK Biobank study, predicted deleterious variants were more frequently found in those self-reporting delayed vs normal age at menarche (OR =1.66; $P$=3.90E-07).

These results indicate that MC3R is a permissive rather than essential factor which influences pubertal timing within a wider polygenic and environmental context.


Reference

1. Lam BYH, Williamson A, Finer S, Day FR, Tadross JA, Gonçalves Soares A, Wade K, Sweeney P, Bedenbaugh MN, Porter DT, Melvin A, Ellacott KLJ, Lippert RN, Buller S, Rosmaninho-Salgado J, Dowsett GKC, Ridley KE, Xu Z, Cimino I, Rimmington D, Rainbow K, Duckett K, Holmqvist S, Khan A, Dai X, Bochukova EG; Genes & Health Research Team; Trembath RC, Martin HC, Coll AP, Rowitch DH, Wareham NJ, van Heel DA, Timpson N, Simerly RB, Ong KK, Cone RD, Langenberg C, Perry JRB, Yeo GS, O'Rahilly S. MC3R links nutritional state to childhood growth and the timing of puberty. *Nature* 2021;599(7885):436-441.


## Basic Research

## 7.9. Understanding the genetic complexity of puberty timing across the allele frequency spectrum


Kentistou KA, Kaisinger LR, Stankovic S, et al.
*Nat Genet*. 2024 Jul;56(7):1397-1411.
doi: 10.1038/s41588-024-01798-4. PMID: 38951643


Brief summary: This multi-ancestry genetic analysis including ~800,000 women, identified 1,080 signals for age at menarche, explaining 11% of trait variance.

Age at menarche is a highly polygenic trait which varies widely among individuals (4-5 years)[1]. Recent genome wide association studies, mostly conducted in subjects of European ancestry, have identified several hundred of loci corresponding to ~ 25% of the hereditability[2-5].

The current study expanded the analysis to 799,845 women, including 166,890 of East Asian ancestry. Women at the top and bottom 1% of polygenic risk exhibited ~11 and ~14-fold higher risks of delayed and precocious puberty, respectively, suggesting that common genetic variants contribute to the risk of rare clinical disorders of extremely early and delayed puberty.

Additionally, authors performed an exome-wide association study on 222,283 European-ancestry women and identified several rare loss-of function variants including *TACR3* and *MKRN3*, two genes previously reported in rare monogenic disorders of puberty. This data shows the lower penetrance of rare deleterious variants in population-based studies compared to patient cohorts.

The researchers clustered the 1,080 age-at menarche signals in the Norwegian Mother, Father and Child Cohort Study ($n$=26,681 children) by their associations with body weight from birth to age 8 years. This approach provided a clear distinction between age at menarche signals that have direct effects on puberty timing or indirect effects by altering early weight gain.

Using GnRH neuron RNAsequencing, authors identified an enrichment for age at menarche associations among genes that are upregulated when GnRH neurons complete their migration and start to make synaptic contacts. The study also highlighted the crucial role of brain G-protein coupled receptors in age at menarche. Twenty-four of 161 brain-expressed GPCRs were implicated in age at menarche. Functional studies identified physical and functional interactions between MCR3 and GPR83, indicating that increased MC3R function through enhanced *GPR83* expression leads to earlier puberty timing.
Some variants were involved in the timing of both menarche and menopause. Interestingly, most variants corresponded to components of the hypothalamic-pituitary-gonadal axes with many of those genes involved in ovarian DNA damage response.

In conclusion, this study doubled the explained variance in age at menarche. Additionally, the authors have developed a common variant polygenic score which will need to be evaluated for determining the risk to develop extreme disorders of puberty timing.


References

1. Wehkalampi K, Silventoinen K, Kaprio J, Dick DM, Rose RJ, Pulkkinen L, Dunkel L. Genetic and environmental influences on pubertal timing assessed by height growth. *Am J Hum Biol.* 2008; 20(4):417-23.
2. Day FR, Thompson DJ, Helgason H, et al.. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet.* 2017; 49(6):834-841.
3. Lunetta KL, Day FR, Sulem P, et al. Rare coding variants and X-linked loci associated with age at menarche. *Nat Commun.* 2015; 6:7756. Erratum in: Nat Commun. 2015; 6:10257.
4. Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.* 2010; 42(12):1077-85.
5. Perry JR, Day F, Elks CE, et al.. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature.* 2014; 514(7520):92-97.


## 7.10. Changes in the bile acid pool and timing of female puberty: potential novel role of hypothalamic TGR5


Vanden Brink H, Vandeputte D, Brito IL, Ronnekleiv OK, Roberson MS, Lomniczi A



*Endocrinology.* 2024 Jul 31:bqae098.

doi: 10.1210/endocr/bqae098. PMID: 39082696


Brief summary: This translational study identifies significant shifts in bile acid composition associated with puberty in female adolescents and rats and identifies a new mechanism of GnRH secretion regulation by bile acid.

Metabolic cues regulate the reactivation of the GnRH system at puberty. The last few years have identified leptin, ghrelin and essential fatty acids as permissive or inhibitory cues regulating GnRH neuron activity[1-4]. The effects of those signalling factors are mostly mediated by the kisspeptin-neurokinin-dynorphin neurons in the arcuate nucleus[5]. Recent studies have shown that the gut microbiome influences metabolic and reproductive health in humans and suggested that regulation of bile acid metabolism by the microbiome might be a mechanism through which environmental factors regulate reproduction[6].

This study reported changes in bile acid composition in the serum of female adolescent and rats throughout puberty. This shift in bile composition was accompanied by a shift in gut microbial composition suggesting that the gut microbiome was the mediator linking bile acid isoforms to pubertal maturation. Authors also showed that the expression of *tgr5*, a gene coding for a bile acid receptor, increased in the female rat arcuate nucleus throughout puberty. The authors showed that TGR5 was expressed by kisspeptin neurons and that bile acids stimulate GnRH secretion from hypothalamic explants through a mechanism involving kisspeptin. Moreover, they showed that overexpression of *tgr5* in the arcuate nucleus leads to early onset of puberty in female rats.

In summary, this study defines a new role for the hepatic-gut-neuroendocrine axis in the control of puberty. Bile acid might provide a key metabolic signal which modulates the timing of puberty and is influenced by the gut microbiome. This suggest a potential role of the microbiome in pathological situations associated with abnormal puberty such as chronic inflammatory bowel diseases or obesity.


References

1. Smith SS, Neuringer M, Ojeda SR. Essential fatty acid deficiency delays the onset of puberty in the female rat. *Endocrinology.* 1989;125(3):1650-1659.
2. Cheung CC, Thornton JE, Nurani SD, Clifton DK, Steiner RA. A Reassessment of Leptin's Role in Triggering the Onset of Puberty in the Rat and Mouse. *Neuroendocrinology.* 2001; 74(1):12-21.
3. Tena-Sempere M. Roles of ghrelin and leptin in the control of reproductive function. *Neuroendocrinology.* 2007; 86(3):229-241.
4. Vazquez MJ, Toro CA, Castellano JM, et al. SIRT1 mediates obesity- and nutrient dependent perturbation of pubertal timing by epigenetically controlling Kiss1 expression. *Nat Commun.* 2018; 9(1):4194.
5. Manfredi-Lozano M, Roa J, Tena-Sempere M. Connecting metabolism and gonadal function: Novel central neuropeptide pathways involved in the metabolic control of puberty and fertility. Connecting metabolism and 25 gonadal function: Novel central neuropeptide pathways involved in the metabolic 26 control of puberty and fertility. *Front Neuroendocrinol.* 2018; 48:37-49.
6. Giampaolino P, Foreste V, Di Filippo C, Gallo A, Mercorio A, Serafino P, Improda FP, Verrazzo P, Zara G, Buonfantino C, Borgo M, Riemma G, Angelis C, Zizolfi B, Bifulco G, Della Corte L. Microbiome and PCOS: State-of-Art and Future Aspects. *Int J Mol Sci.* 2021; 22(4):2048.

## 7.11. Glutamate neurotransmission from leptin receptor cells is required for typical puberty and reproductive function in female mice

Sáenz de Miera C, Bellefontaine N, Allen SJ, Myers MG, Elias CF
*Elife*. 2024 Jul 15;13:RP93204.
doi: 10.7554/eLife.93204. PMID: 39007235.
https://elifesciences.org/articles/93204

Brief summary: This study used chemogenetics and transgenic mouse models to show that glutamatergic neurotransmission in leptin responsive neurons in the premammillary nucleus is required for normal puberty and ovulation.

Puberty and the acquisition of reproductive functions result from the reawakening of a complex neuroendocrine machinery eventually leading to the activation of GnRH secretion. Metabolic cues play a crucial role in regulating this system with leptin being one of the main actors of the crosstalk between energy balance and reproduction[1]. Recent data showed that the premammilary nucleus (PMv) in the hypothalamus is the relay of nutritional state information to the GnRH neurons[2,3] as leptin does not act directly onto GnRH neurons. The current study further characterizes the hypothalamic circuits involved in the modulation of GnRH secretion by metabolic cues.

The authors first showed that stimulation of neurons expressing the leptin receptor in the PMv using stimulatory form of designer receptor exclusively activated by designer drugs (DREADDS) approaches induced LH release. The data also indicate that females with deletion of vGlut2, a glutamate transporter, in neurons expressing the leptin receptor showed normal vaginal opening but delayed age at first estrus, disrupted estrous cycles, increased gonadotropin-releasing hormone (GnRH) concentration in the axon terminals and disrupted LH secretion, suggesting disruption of GnRH secretion. To evaluate if glutamate neurotransmission is required for leptin-induced pubertal development, the researchers produced a dual-floxed mouse model in which a stereotaxic delivery of a viral vector restored leptin receptor expression while deleting VGLUT2 in the PMv. Using this approach, they showed that leptin action on pubertal development requires Vglut2.

Together, these data indicate that glutamate signalling in leptin sensitive neurons in the PMv is required for normal puberty and ovulation. The relationship between kisspeptin neurons and these neuronal populations in the PMv remains to be determined.

References


1. Anderson GM, Hill JW, Kaiser UB, Navarro VM, Ong KK, Perry JRB, Prevot V, Tena-Sempere M, Elias CF. Metabolic control of puberty: 60 years in the footsteps of Kennedy and Mitra's seminal work. *Nat Rev Endocrinol.* 2024; 20(2):111-123.
2. Donato J Jr, Cravo RM, Frazão R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr, Coppari R, Zigman JM, Elmquist JK, Elias CF. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. *J Clin Invest.* 2011; 121(1):355-68.
3. Ross RA, Leon S, Madara JC, Schafer D, Fergani C, Maguire CA, Verstegen AM, Brengle E, Kong D, Herbison AE, Kaiser UB, Lowell BB, Navarro VM. PACAP neurons in the ventral premammillary nucleus regulate reproductive function in the female mouse. *Elife*. 2018; 7:e35960


## 7.12. Stress during pubertal development affects female sociosexual behavior in mice

Bentefour Y, Bakker J
*Nat Commun*. 2024 Apr 30;15(1):3610.
doi: 10.1038/s41467-024-47300-w. PMID: 38688927
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11061123/

Brief summary: This study demonstrates that stress during puberty in mice leads to a permanent disruption in female sexual behavior, specifically affecting sexual performance and disrupted estrous cycles.

Puberty is critical for the development of the female brain and sexual behavior[1,2]. The neural circuits involved in the establishment of the control of sexual behavior are still poorly understood.

The authors found that female mice exposed to pubertal stress displayed decreased sexual receptivity and disrupted estrous cycles, spending less time in proestrus and estrus phases. These pubertal effects were associated with reduced activation of neuronal nitric oxide synthase (nNOS) neurons in the ventrolateral part of the ventromedial hypothalamus (VMHvl). These neurons have been recently described as necessary for the

expression and the modulation of female sexual behavior, with nNOS neurons being a downstream target of kisspeptin[3,4]. Using fiber photometry, theyshowed that these VMHvl nNOS neurons responded typically to male olfactory cues, but this response was significantly blunted in pubertally stressed females.

Additionally, the study explored the hormonal mechanisms underlying these changes, finding that pubertal stress did not affect circulating levels of estradiol or progesterone. This suggested that the observed sexual dysfunction was not hormone-driven. The researchers attempted to rescue sexual behavior in stressed females by administering a nitric oxide (NO) donor. This treatment partially restored sexual performance, further highlighting the role of nNOS neurons in the VMHvl in regulating female sexual behavior.

This study provides valuable insights into the neural mechanisms underlying the effects of pubertal stress on female sexual behavior and emphasizes the critical role of the VMHvl nNOS neurons and the importance of proper neural circuit development during puberty.

References


1. Bakker, J., Honda, S. I., Harada, N. & Balthazart, J. The aromatase knock-out mouse provides new evidence that estradiol is required during development in the female for the expression of sociosexual behaviors in adulthood. *J. Neurosci.* 2002; 22, 9104–9112.
2. Brock, O., Baum, M. J. & Bakker, J. The development of female sexual behavior requires prepubertal estradiol. *J. Neurosci.* 2011; 31, 5574–5578.
3. Bentefour, Y. & Bakker, J. Kisspeptin signaling and nNOS neurons in the VMHvl modulate lordosis behavior but not mate preference in female mice. *Neuropharmacology* 2021; 198, 108762.
4. Silva MSB, Decoster L, Trova S, Mimouni NEH, Delli V, Chachlaki K, Yu Q, Boehm U, Prevot V, Giacobini P. Female sexual behavior is disrupted in a preclinical mouse model of PCOS via an attenuated hypothalamic nitric oxide pathway. *Proc Natl Acad Sci U S A.* 2022;119(30):e2203503119.
5. Female sexual behavior is disrupted in a preclinical mouse model of PCOS via an attenuated hypothalamic nitric oxide pathway. *Proc. Natl Acad. Sci. USA* 2022; 119, e2203503119.


## 7.13. Metabolic control of puberty: 60 years in the footsteps of Kennedy and Mitra's seminal work


Anderson GM, Hill JW, Kaiser UB, Navarro VM, Ong KK, Perry JRB, Prevot V, Tena-Sempere M, Elias CF

*Nat Rev Endocrinol*. 2024 Feb;20(2):111-123.

doi: 10.1038/s41574-023-00919-z. PMID: 38049643


Brief summary: This review summarizes the role of macronutrients and hormones which regulate energy balance and sexual maturation by conveying energy availability information to the GnRH system.

30 years ago, Kennedy and Mitra were the first to use translational studies to show that nutrition is a key factor regulating puberty timing[1]. Their seminal protocol modified the size of rat litters to affect weight increase and showed that nutritional status affected pubertal maturation[1]. This study has paved the way for translational, clinical and epidemiological studies illustrating the importance of the regulation of reproductive maturation by energy accessibility. Human examples of this interaction between energy balance and pubertal onset cover earlier puberty associated with overweight as well as delayed puberty in conditions of undernutrition[2,3].

This review describes recent progress made in understanding the neuronal and glial circuitry regulating the reactivation of the GnRH network at the time of puberty[4] as well as epigenetic mechanisms such as the metabolic sensor SIRT1 which links early nutritional status to puberty[5]. Population genetics as well as studies of individual patients with extreme perturbation of weight or puberty have also identified new key players in the crosstalk between sexual maturation and energy balance such as Delta-like homologue 1[6] or melanocortin receptor 3[7]. As discussed by the authors, fully understanding the mechanisms that connect obesity and earlier onset of puberty deserves additional research efforts.

References


1. Kennedy GC, Mitra J. Body weight and food intake as initiating factors for puberty in the rat. *J Physiol.* 1963;166(2):408-18. doi: 10.1113/jphysiol.1963.sp007112. PMID: 14031944; PMCID: PMC1359337.
2. Reinehr, T. & Roth, C. L. Is there a causal relationship between obesity and puberty? *Lancet Child. Adolesc. Health 2019;* 3, 44–54 (2019).
3. Argente J, Dunkel L, Kaiser UB, Latronico AC, Lomniczi A, Soriano-Guillén L, Tena-Sempere M. Molecular basis of normal and pathological puberty: from basic mechanisms to clinical implications. *Lancet Diabetes Endocrinol.* 2023;11(3):203-216. doi: 10.1016/S2213-8587(22)00339-4.
4. Avendano, M. S., Vazquez, M. J. & Tena-Sempere, M. Disentangling puberty: novel neuroendocrine pathways and mechanisms for the control of mammalian puberty. *Hum. Reprod.* Update 2017; 23, 737–763.

5. Vazquez MJ, Toro CA, Castellano JM, Ruiz-Pino F, Roa J, Beiroa D, Heras V, Velasco I, Dieguez C, Pinilla L, Gaytan F, Nogueiras R, Bosch MA, Rønnekleiv OK, Lomniczi A, Ojeda SR, Tena-Sempere M. SIRT1 mediates obesity- and nutrient-dependent perturbation of pubertal timing by epigenetically controlling Kiss1 expression. *Nat Commun.* 2018 Oct 10;9(1):4194. doi: 10.1038/s41467-018-06459-9. PMID: 30305620; PMCID: PMC6179991.
6. Dauber A, Cunha-Silva M, Macedo DB, Brito VN, Abreu AP, Roberts SA, Montenegro LR, Andrew M, Kirby A, Weirauch MT, Labilloy G, Bessa DS, Carroll RS, Jacobs DC, Chappell PE, Mendonca BB, Haig D, Kaiser UB, Latronico AC. Paternally Inherited DLK1 Deletion Associated With Familial Central Precocious Puberty. *J Clin Endocrinol Metab.* 2017 May 1;102(5):1557-1567. doi: 10.1210/jc.2016-3677. PMID: 28324015; PMCID: PMC5443333.
7. Lam BYH, Williamson A, Finer S, Day FR, Tadross JA, Gonçalves Soares A, Wade K, Sweeney P, Bedenbaugh MN, Porter DT, Melvin A, Ellacott KLJ, Lippert RN, Buller S, Rosmaninho-Salgado J, Dowsett GKC, Ridley KE, Xu Z, Cimino I, Rimmington D, Rainbow K, Duckett K, Holmqvist S, Khan A, Dai X, Bochukova EG; Genes & Health Research Team; Trembath RC, Martin HC, Coll AP, Rowitch DH, Wareham NJ, van Heel DA, Timpson N, Simerly RB, Ong KK, Cone RD, Langenberg C, Perry JRB, Yeo GS, O'Rahilly S. MC3R links nutritional state to childhood growth and the timing of puberty. *Nature.* 2021; 599(7885):436-441. doi: 10.1038/s41586-021-04088-9. PMID: 34732894; PMCID: PMC8819628.

# 8. Adrenals

Svetlana Lajic[1], George Paltoglou[2*], Leif Karlsson[1*], Chamila Balagamage[3], Evangelia Charmandari[2,4]

*Equal author contribution

[1]Department of Womeńs and Childreńs Health, Pediatric Endocrinology Unit Karolinska University Hospital/ Karolinska Institutet, 171 76 Stockholm, Sweeden; [2]Division of Endocrinology, Metabolism and Diabetes, First Department of Pediatrics, National and Kapodistrian University of Athens Medical School, 'Aghia Sophia' Children's Hospital, Athens, 11527, Greece; [3]Division of Endocrinology, Metabolism and Diabetes, Birmingham Children's Hospital, Birmingham, B4 6NH, United Kingdom; and [4]Division of Endocrinology and Metabolism, Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, 11527, Greece.

Author for Correspondence: Evangelia Charmandari, MD, MSc, PhD, MRCP(UK), CCST(UK) Professor of Pediatric and Adolescent Endocrinology First Department of Pediatrics National and Kapodistrian University of Athens Medical School 'Aghia Sophia' Children's Hospital Thivon and Papadiamantopoulou Street Athens, 11527, Greece Tel/Fax: +30-213-2013 384 Email: evangelia.charmandari@google mail.com

## Preface

For this year's chapter, we searched the PubMed for articles on 'adrenal' or 'steroidogenesis' published in English between June 1, 2023 and July 31, 2024. Our search yielded > 7,000 citations. We examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we avoided topics that were discussed in the Yearbook 2023, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) Fatty acid desaturase 2 determines the lipidomic landscape and steroidogenic function of the adrenal gland; ii) Mitochondrial cytochrome P450 1B1 is involved in pregnenolone synthesis in human brain cells; iii) Ultradian hydrocortisone replacement alters neuronal processing, emotional ambiguity, affect and fatigue in adrenal insufficiency; iv) High-resolution daily profiles of tissue adrenal steroids by portable automated collection; v) Pediatric Cushing syndrome: a prospective, multisite, observational cohort study; vi) Management of phaeochromocytoma and paraganglioma in patients with germline SDHB pathogenic variants: an international expert Consensus statement; vii) Phase 3 clinical trial of Crinecerfont in children with Congenital Adrenal Hyperplasia; and viii) The Role of Interferon-γ in Autoimmune Polyendocrine Syndrome Type 1.

## Mechanism of the Year

### 8.1. Fatty acid desaturase 2 determines the lipidomic landscape and steroidogenic function of the adrenal gland

Witt A, Mateska I, Palladini A, Sinha A, Wölk M, Harauma A, Bechmann N, Pamporaki C, Dahl A, Rothe M, Kopaliani I, Adolf C, Riester A, Wielockx B, Bornstein SR, Kroiss M, Peitzsch M, Moriguchi T, Fedorova M, Grzybek M, Chavakis T, Mirtschink P, Alexaki VI

*Sci Adv.* 2023; 9(29): eadf6710.

https://pubmed.ncbi.nlm.nih.gov/37478183/

Brief Summary: This study demonstrates that FADS2 is a major regulator of steroidogenesis in the adrenal gland due to its key role in shaping the lipidomic landscape of adrenocortical cells.

Comment: The corticosteroids aldosterone and cortisol are produced in the zona glomerulosa and zona fasciculata of the adrenal cortex, respectively (1, 2). Excess production of aldosterone in primary

hyperaldosteronism results in resistant hypertension and cardiovascular disease (1), while hypercortisolism leads to several comorbidities, including central obesity, impaired glucose tolerance and Type 2 diabetes (T2D), dyslipidemia and hypertension (3). Obesity is associated with moderately increased glucocorticoid production, which exacerbates central obesity, hyperlipidemia, hypertension and cardiovascular disease risk (4–8). However, the mechanisms underlying increased corticoid production in obesity are not fully understood.

Corticosteroid synthesis in the adrenal cortex requires the release of cholesterol from lipid droplets, where it is stored in the form of cholesterol esters (CE), and its translocation into mitochondria via the steroidogenic acute regulatory (StAR) protein, which is the rate-limiting step of steroidogenesis. Once inside the mitochondria, cholesterol is used for steroidogenesis through a series of enzymatic steps.

This study investigated the impact of mitochondrial membrane lipids on steroidogenesis in adrenocortical cells, and in particular to which extent the lipidomic landscape of the adrenal gland affects its steroidogenic function. It analyzed the adrenal lipidome of lean and obese mice and found that a high content of phospholipids in longer and more unsaturated lipids is associated with increased steroidogenesis in obese mice. Arachidonic acid (ARA) is a particularly abundant acyl chain in adrenal phospholipids and is increased with obesity. Inhibition of fatty acid desaturase 2 (FADS2), the rate-limiting enzyme of polyunsaturated fatty acid (PUFA) synthesis, transformed the mitochondrial lipidome, inhibited cholesterol import, and diminished steroidogenesis. FADS2 is highly expressed in the adrenal gland compared to other tissues and is increased in the adrenal gland of obese animals. FADS2 deficiency in mice impaired mitochondrial structure in adrenocortical cells and reduced corticosterone and aldosterone production. Conversely, FADS2 expression was up-regulated in the adrenal glands of obese mice and in aldosterone-producing adenomas compared to non-active adenomas (producing low amounts of aldosterone) and non-tumorous adrenocortical tissue of patients, while FADS2 inhibition reduced corticoid concentrations in obese mice.

These data collectively identify FADS2 as a major regulator of adrenal gland steroidogenesis due to its key role in shaping the lipidomic landscape of adrenocortical cells. Moreover, the adrenal lipidome can be modulated by icosapent ethyl dietary supplementation, which consequently influences corticoid production. This finding endorses the evaluation of icosapent ethyl dietary supplementation as a means to regulate cortisol and aldosterone concentrations in obesity. Ethyl eicosapentaenoic acid (E-EPA, icosapent ethyl) is made from the omega-3 fatty acid eicosapentaenoic acid (EPA) and is often used to treat dyslipidemia and hypertriglyceridemia. Finally, these data indicate a clear correlation between FADS2 expression and steroidogenic capacity in the human adrenal gland. The role of FADS2–mediated lipidomic changes in adrenocortical tumorigenesis should be further investigation.

References


1. Feldman RD. Aldosterone and blood pressure regulation: recent milestones on the long and winding road from electrocortin to KCNJ5 GPER, and beyond. *Hypertension*. 2014; 63(1): 19-21.
2. Lightman SL, Birnie MT, Conway-Campbell BL. Dynamics of ACTH and Cortisol Secretion and Implications for Disease. *Endocr Rev*. 2020; 41(3): bnaa002.
3. Koch CA. Cushing syndrome and glucocorticoid excess, in Disorders of Blood Pressure Regulation: Phenotypes, mechanisms, therapeutic options, A. Berbari, G. Mancia, Eds, (Springer, 2018), pp. 481–512.
4. Calhoun DA, Sharma K. The role of aldosteronism in causing obesity-related cardiovascular risk. *Cardiol Clin.* 2010; 28(3): 517-27.
5. Swierczynska MM, Mateska I, Peitzsch M, Bornstein SR, Chavakis T, Eisenhofer G, Lamounier-Zepter V, Eaton S. Changes in morphology and function of adrenal cortex in mice fed a high-fat diet. *Int J Obes (Lond).* 2015; 39(2): 321-30.
6. Hofmann A, Peitzsch M, Brunssen C, Mittag J, Jannasch A, Frenzel A, Brown N, Weldon SM, Eisenhofer G, Bornstein SR, Morawietz H. Elevated Steroid Hormone Production in the db/db Mouse Model of Obesity and Type 2 Diabetes. *Horm Metab Res.* 2017; 49(1): 43-49.
7. Galitzky J, Bouloumié A. Human visceral-fat-specific glucocorticoid tuning of adipogenesis. *Cell Metab.* 2013; 18(1): 3-5.
8. Roberge C, Carpentier AC, Langlois MF, Baillargeon JP, Ardilouze JL, Maheux P, Gallo-Payet N. Adrenocortical dysregulation as a major player in insulin resistance and onset of obesity. *Am J Physiol Endocrinol Metab.* 2007; 293(6): E1465-78.


## Other New Mechanisms

## 8.2. Mitochondrial cytochrome P450 1B1 is involved in pregnenolone synthesis in human brain cells


Lin YC, Cheung G, Zhang Z, Papadopoulos V



*J Biol Chem*. 2023; 299(8): 105035.
https://pubmed.ncbi.nlm.nih.gov/37442234/

Brief Summary: This study investigated the synthesis pathway of pregnenolone (PREG) in the central nervous system. It identified the P450 enzyme CYP1B1 to be responsible for the production of PREG in human glial cells.

Comment: Steroids have important functions in the central and peripheral nervous systems, including modulation of behavior, pain, stress and inflammation. Steroids in the brain can be produced *de novo*, as well as transported from peripheral tissues (1). Locally produced steroids have genomic actions, and also induce allosteric modulation of neurotransmittor receptors (non-genomic actions).

Pregnenolone (PREG) is an active neurosteroid, and the precursor of other neurosteroids. It has roles in memory formation, neuroplasticity, neuroprotection and has anti-inflammatory properties (2). In clinical trials, PREG shows therapeutic potential in schizophrenia, bipolar depression and chronic pain disorders. However, the biosynthetic pathway of PREG in the brain is unknown. This study investigated the synthesis of PREG in the central nervous system (CNS).

Previously, these authors found very low mRNA levels in human brain of CYP11A1 and undetectable protein levels of CYP11A1, the enzyme responsible for PREG biosynthesis in the adrenal gland (3), indicating that another enzyme has this role in the brain. They used qRT-PCR in human glial cell lines to screen for other CYP450s that might synthesize PREG and identified CYP27A1, CYP1A1 and CYP1B1 as potential candidates. They excluded CYP27A1, since its inhibition or siRNA knock-down did not affect the levels of PREG.

Of the other two candidate enzymes, CYP1B1 but not CYP1A1 was found to produce PREG in glial cells but not in adrenal cells. They came to this conclusion with a series of experiments utilizing enzyme inhibitors, siRNA knock-down experiments and over-expression of CYP1B1 in glial cells. To understand from which substrate PREG is formed, they transfected the cells with cholesterol (LDL). In the cell line where CYP1B1 protein was localized to the mitochondria, there was a dose-dependent increase in PREG production, showing that PREG synthesis can use cholesterol as a substrate.

In conclusion, this study identifies the synthesis pathway of PREG in the CNS, involving the P450 enzyme CYP1B1 and both hydroxycholesterol and cholesterol as substrates. This pathway differs from classical adrenal steroidogenesis. Consideration should be taken when using drugs that pass the blood-brain barrier and compete with the CYP1B1 substrates since this could affect brain function.

References


1. Baulieu EE. Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology*. 1998; 23(8): 963-87.
2. Lin YC, Cheung G, Espinoza N, Papadopoulos V. Function, regulation, and pharmacological effects of pregnenolone in the central nervous system. *Curr. Opin. Endocr. Metab. Res.* 2022; 22: 100310
3. Lin YC, Cheung G, Porter E, Papadopoulos V. The neurosteroid pregnenolone is synthesized by a mitochondrial P450 enzyme other than CYP11A1 in human glial cells. *J Biol Chem.* 2022; 298(7): 102110.


## Important for Clinical Practice

## 8.3. European Society of Endocrinology and Endocrine Society Joint Clinical Guideline: Diagnosis and Therapy of Glucocorticoid-induced Adrenal Insufficiency


Beuschlein F, Else T, Bancos I, Hahner S, Hamidi O, van Hulsteijn L, Husebye ES, Karavitaki N, Prete A, Vaidya A, Yedinak C, Dekkers OM



*J Clin Endocrinol Metab*. 2024; 109(7): 1657-1683.

https://pubmed.ncbi.nlm.nih.gov/38724043/


Brief Summary: This article presents the joint European Society of Endocrinology and Endocrine Society clinical guideline on the diagnosis and treatment of Glucocorticoid (GC)-induced adrenal insufficiency

Comment: The prevalence of oral glucocorticoid (GC) use is ~1% in adults (1). The risk for glucocorticoid-induced adrenal insufficiency is evident. These guidelines provide both endocrinologists and general practitioners with guidance to manage such patients, regarding tapering of GC doses, assessing adrenal function and providing stress dosing. Any route of GC administration has potential to suppress the hypothalamic-

pituitary-adrenal (HPA) axis, and suppression is dependent on the dose, duration of treatment and the potency of the GC. A meta-analysis suggested the risk of biochemical GC-induced adrenal insufficiency (AI) was 4.2% with nasal administration, 49% with oral administration, and 52% with intra-articular administration (2). However, biochemical AI is not the same as clinically-evident AI. Symptoms of AI were reported in only 10% of patients with biochemical AI (2).

*Clinical question I:* What is the incidence of recovery of HPA-axis function in patients with GC-induced AI? Meta-analysis (2) showed that the prevalence of AI decreased from 39% to 15% by 4 weeks after discontinuation of short-term GC therapy (< 4 weeks treatment). With moderate doses and long-term GC therapy (> 1 y) the prevalence of AI decreased from 56% to 25% by 6 months after GC discontinuation.

*Clinical question II:* Which clinical/biochemical parameters predict recovery of HPA-axis function in patients with GC-induced AI? Higher cortisol increments on standard ACTH stimulation testing (just after GC discontinuation) were observed in patients who recovered vs. did not recover (219 vs. 99 nmol/L) (3). Patients who recovered also had higher ambulatory morning cortisol concentrations (286 vs. 186 nmol/L) (4).

*Clinical question III:* What is the optimal tapering scheme? It is safe to stop GC use abruptly after short term use (< 4 weeks) of high dose GC-treatment without testing. With long-term GC use, rapid tapering can be used to reduce from supra-physiologic doses, followed by slower taper once on physiologic doses. HPA recovery is possible while on physiologic doses (4-6 mg PRED or 15-25 mg HC). Patients taking long-acting GC should be switched to short-acting when possible.

*Clinical question IV:* What is the diagnostic accuracy of a morning cortisol value vs. 250 mcg ACTH test? Morning cortisol values >300 nmol/L indicate a normal HPA-axis. Morning cortisol values < 150 nmol/L indicate that GC treatment should continue, with retesting after a few months. For morning cortisol values between 150-300 nmol/L, physiologic GC dosing should continue, with a retest after weeks-months.

The authors suggest that routine dynamic testing is not necessary. If dynamic testing is performed, it should be done 24-h after stopping GC treatment to avoid assay interference. If the HPA axis has not recovered after 1 year on physiologic GC doses, patients should be evaluated by an endocrinologist. Patients with current or recent GC use who have not undergone biochemical testing for AI should have stress dose GC coverage when they undergo stress.

References


1. Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. *Rheumatology (Oxford).* 2011; 50(11): 1982-90.
2. Broersen LH, Pereira AM, J½rgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab.* 2015; 100(6): 2171-80.
3. Baek JH, Kim SK, Jung JH, Hahm JR, Jung J. Recovery of Adrenal Function in Patients with Glucocorticoids Induced Secondary Adrenal Insufficiency. *Endocrinol Metab (Seoul).* 2016; 31(1): 153-60.
4. Leong SH, Shander S, Ratnasingam J. Predicting Recovery of the Hypothalamic-Pituitary-Adrenal Axis After Prolonged Glucocorticoid Use. *Endocr Pract.* 2018; 24(1): 14-20.


## 8.4. Incidence and risk factors for adrenal crisis in pediatric-onset adrenal insufficiency: a prospective study


Hosokawa M, Ichihashi Y, Sato Y, Shibata N, Nagasaki K, Ikegawa K, Hasegawa Y, Hamajima T, Nagamatsu F, Suzuki S, Numakura C, Amano N, Sasaki G, Nagahara K, Soneda S, Ariyasu D, Maeda M, Kamasaki H, Aso K, Hasegawa T, Ishii T



*J Clin Endocrinol Metab*. 2024; 109(8): e1602-e1607.
https://pubmed.ncbi.nlm.nih.gov/38128002/


Brief Summary: This study examined the incidence and risk factors for adrenal crisis (AC) in patients with pediatric-onset adrenal insufficiency (AI). AC occurs in a substantial number of children with AI, particularly in younger children due to their high number of infections.

Comment: Adrenal crisis (AC) is a life-threatening complication in patients with adrenal insufficiency (AI) (1-4). The clinical features of AC are often nonspecific, and it may be unrecognized, especially in children. This can

delay appropriate glucocorticoid treatment, and potentially result in death or irreversible brain damage (1, 5). Therefore, it is important to identify at-risk patients and intervene to prevent AC.

This multicenter, prospective cohort of patients diagnosed with AI at age $\leq$ 15 years in Japan, examined the incidence and risk factors for AC. Previous studies were retrospective. 349 patients (164 male, 185 female; median age: 14.3 years, interquartile range: 8.5-21.2 years) from 20 pediatric endocrinology clinics were recruited, and followed for 2.8 years (IQR 2.2-3.3 years). 213 patients (61%) had primary AI and 136 (39%) had secondary AI.

During the study period (2018-2022), physicians collected information on: height, weight, blood pressure, dose of glucocorticoids (GC) and mineralocorticoids (MC), presence or absence of fever, vomiting, diarrhea, surgery or trauma requiring stress dose of hydrocortisone (HC), and im or iv administration of HC. Overall, 41 episodes of AC occurred in 31 patients. In 40 events, iv HC was given at a medical institution; in only 1 event im HC was given at home (im HC self-injection was introduced in Japan in April 2020). The incidence of AC was 4.27 per 100 person years (PY). Among patients aged $<$ 20 years, the incidence of AC was 6.58 per 100 PY. Risk factors for AC were: younger age at enrollment, and higher number of infections. Female sex, primary AI and GC dose were not significant risk factors.

This is the largest study to date on the incidence of AC in children with AI. The findings concur with previous retrospective studies and suggest that AC occurs in a substantial number of pediatric patients with AI. Particular attention should be given to younger children due to the increased incidence of infections.

References


1. Allolio B. Extensive expertise in endocrinology. Adrenal crisis. *Eur J Endocrinol.* 2015; 172(3): R115-R124.
2. Eyal O, Levin Y, Oren A, Zung A, Rachmiel M, Landau Z, Schachter-Davidov A, Segev-Becker A, Weintrob N. Adrenal crises in children with adrenal insufficiency: epidemiology and risk factors. *Eur J Pediatr.* 2019; 178(5): 731-738.
3. Hahner S, Loeffler M, Bleicken B, Drechsler C, Milovanovic D, Fassnacht M, Ventz M, Quinkler M, Allolio B. Epidemiology of adrenal crisis in chronic adrenal insufficiency: the need for new prevention strategies. *Eur J Endocrinol.* 2010; 162(3): 597-602.
4. Hahner S, Spinnler C, Fassnacht M, Burger-Stritt S, Lang K, Milovanovic D, Beuschlein F, Willenberg HS, Quinkler M, Allolio B. High incidence of adrenal crisis in educated patients with chronic adrenal insufficiency: a prospective study. *J Clin Endocrinol Metab.* 2015; 100(2): 407-416.
5. Ishii T, Adachi M, Takasawa K, Okada S, Kamasaki H, Kubota T, Kobayashi H, Sawada H, Nagasaki K, Numakura C, Harada S, Minamitani K, Sugihara S, Tajima T. Incidence and characteristics of adrenal crisis in children younger than 7 years with 21-hydroxylase deficiency: a nationwide survey in Japan. *Horm Res Paediatr.* 2018; 89(3): 166-171.


## 8.5. High-resolution daily profiles of tissue adrenal steroids by portable automated collection


Upton TJ, Zavala E, Methlie P, Kämpe O, Tsagarakis S, Øksnes M, Bensing S, Vassiliadi DA, Grytaas MA, Botusan IR, Ueland G, Berinder K, Simunkova K, Balomenaki M, Margaritopoulos D, Henne N, Crossley R, Russell G, Husebye ES, Lightman SL



*Sci Transl Med*. 2023; 15(701): eadg8464.

https://pubmed.ncbi.nlm.nih.gov/37343084/


Brief Summary: This paper describes a novel ambulatory fraction collector that can be used with microdialysis to obtain high resolution steroid profiles over a 24-hour period.

Comment: The natural circadian and ultradian secretion of adrenal hormones makes single timepoint measurements of these hormones uninformative for clinical decision making (1-3). Repeated sampling during the day requires admission into an atypical, and often disruptive, clinical setting, particularly if overnight sampling is attempted. These authors addressed this issue by developing an ambulatory fraction collector that can be used with microdialysis to collect samples continuously throughout the day without interfering with daily activities.

They enrolled 214 healthy individuals to collect 24-hour steroid profiles, using this new ambulatory sampling method for cortisol, cortisone, corticosterone, 18-hydroxycortisol, aldosterone, tetrahydrocortisol, allo-tetrahydrocortisol and dehydroepiandrosterone sulfate. The study provides data on the normal physiology and dynamics of tissue steroids, as well as normal reference values. These can be used further to define changes associated with adrenal disease, alongside a powerful methodology to improve personalized replacement therapy.

References

1. Oster H, Challet E, Ott V, Arvat E, de Kloet ER, Dijk DJ, Lightman S, Vgontzas A, Van Cauter E. The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. *Endocr Rev.* 2017; 38(1): 3-45.
2. Russell G, Lightman S. The human stress response. *Nat Rev Endocrinol.* 2019; 15(9): 525-534.
3. Horrocks PM, Jones AF, Ratcliffe WA, Holder G, White A, Holder R, Ratcliffe JG, London DR. Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clin Endocrinol (Oxf).* 1990; 32(1): 127-34.

## 8.6. Glucose. pattern in children with classical congenital adrenal hyperplasia: evidence from continuous glucose monitoring

Galderisi A, Kariyawasam D, Stoupa A, Quoc AN, Pinto G, Viaud M, Brabant S, Beltrand J, Polak M, Samara-Boustani D

*Eur J Endocrinol*. 2023; 189(5): K19-K24.
https://pubmed.ncbi.nlm.nih.gov/37952170/

Brief Summary: This study investigated daily glucose patterns in young children with classic congenital adrenal hyperplasia (CAH) and their relation with hormonal circadian rhythm.

Comment: The aim of treatment in classical CAH is to provide adequate glucocorticoid and – when necessary – mineralocorticoid replacement to prevent adrenal crises and suppress excess adrenal androgen production. Glucocorticoids regulate glucose homeostasis, and patients with classical CAH are at risk of severe hypoglycemia during stress and infections (1). However, mimicking the physiologic, circadian rhythm in cortisol secretion is impossible due to the pharmacokinetics of typical, immediate-release, hydrocortisone (HC) tablets (2). Hence, children receiving HC for CAH may be exposed to hypoglycemic periods during the day.

This study used subcutaneous continuous glucose monitoring (CGM) to assess the prevalence of hypoglycemia in young children with CAH (n = 11, 1-6 years) and to identify any relationship with hormonal circadian rhythm. Cortisol and ACTH samples were collected for a 24-hour inpatient admission. Children with CAH were more often exposed to asymptomatic hypoglycemia during the night and morning than age-matched healthy controls (3). Daily cortisol patterns paralleled CGM levels. As prolonged and frequent exposure to hypoglycemia may adversely affect neurodevelopment, these findings suggest CGM may be a supportive tool in the clinical management of young children with CAH, to guide HC dose adjustments.

References

1. Odenwald B, Nennstiel-Ratzel U, Dörr H, Schmidt H, Wildner M, Bonfig W. Children with classic congenital adrenal hyperplasia experience salt loss and hypoglycemia: evaluation of adrenal crises during the first 6 years of life. *Eur J Endocrinol.* 2016; 174(2): 177-186.
2. Auer M, Nordenström A, Lajic S, Reisch N. Congenital adrenal hyperplasia. *Lancet.* 2023; 401(10372): 227-244.
3. DuBose S, Kanapka L, Bradfield B, Sooy M, Beck R, Steck A. Continuous glucose monitoring profiles in healthy, nondiabetic young children. *J Endocr Soc.* 2022; 6(6): bvac060.

## 8.7. Parental concerns about genital differences in children with congenital adrenal hyperplasia persist regardless of the selected intervention

Alderson J, Thornton M, Skae M, Jones J, Nicoll N, Harcourt D, Woodward M, Crowne EC

*J Sex Med*. 2024; 21(5): 361-366.
https://pubmed.ncbi.nlm.nih.gov/38481013/

Brief Summary: This qualitative study investigated parental communication with their daughters regarding variation in clitoral size related to severity of classical congenital adrenal hyprplasia (CAH). The authors conducted semi-structured in-person interviews with 24 parents of children with a specific genital difference, without direct exploration of parental values regarding the clitoris or the application of adequate psychosocial care.

Comment: CAH due to 21-hydroxylase deficiency (21-OHD) requires life-long medical management of adrenal insufficiency and adrenal androgen excess. 46,XX children with 21-OHD have altered genital morphology, including clitoral enlargement, due to high fetal androgen secretion *in utero*, and this condition is therefore recognized by most medical experts to be a 'difference of sex development' (DSD). Childhood clitoral surgery

has been questioned owing to concerns about negative long-term effects on clitoral sensitivity and sexual function (1). As reported in previous studies, the parents interviewed here worried about their child's sexual future, and expressed considerable hesitancy about educating the child and facilitating a growing awareness of their development at birth. (2) (3).

This study focused on clitoral variation as a means of exploring parents' communication and barriers in the management of children's atypical genital appearance. Parents were led by professionals to an "Obvious Choice" regarding accepting or avoiding early childhood clitoral surgery, after which most parents perceived their child's genitals to be "Still Different". The impact of their child's genital difference remained a psychological and practical "Parental Burden," and most parents alluded to a belief that they should prevent their child from knowing about her genital difference because "Ignorance is Bliss." Almost all participants referenced their belief in benevolent ignorance and the burden of responsibility caused by feeling able to maintain the child's lack of awareness, poor knowledge, and pre-sexual life temporary state of ignorance. This approach is controversial given that adults with experience have repeatedly interpreted parental inability to talk about their bodily difference as a sign of unspeakable shame (4). Exclusion of CAH from DSD is controversial, although this would not eliminate the ongoing bioethical debate on surgical alteration of the clitoris in childhood (5).

An important dimension of multi-professional care is facilitating patient self-knowledge. This study suggests that DSD services may not be fully aware of the lack of information available to children if the responsibility for education falls on their parents. Health professionals share responsibility for the child well-being via partnership and direct support of parents. These findings suggest that neither childhood genital surgery, nor its absence, singularly eradicates parental perception of difference or removes parental concerns. Healthcare services must support parental ability to engage in essential communication with their children on topics such as clitoral size variation related to neonatal CAH.

References


1. Crouch NS, Liao LM, Woodhouse CR, Conway GS, Creighton SM. Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia. *J Urol.* 2008; 179(2): 634-8.
2. Lundberg T, Lindstrom A, Roen K, Hegarty P. From Knowing Nothing to Knowing What, How and Now: Parents' Experiences of Caring for their Children with Congenital Adrenal Hyperplasia. *J Pediatr Psychol.* 2017; 42(5): 520-9.
3. Thornton M, Harcourt D, Deave T, Kiff J, Williamson H. "Have We Done Enough?" A Cross-condition Exploration of the Experiences of Parents Caring for A Child with an Appearance-affecting Condition or Injury. *Dev Neurorehabil.* 2021; 24(6): 418-28.
4. Alderson J, Madill A, Balen A. Fear of devaluation: understanding the experience of intersexed women with androgen insensitivity syndrome. *Br J Health Psychol.* 2004; 9(Pt 1): 81-100.
5. Gonzalez R, Ludwikowski BM. Is It Beneficial to Patients to Include Congenital Adrenal Hyperplasia (CAH) Among the Disorders of Sex Development (DSD)? *Front Pediatr.* 2018; 6: 344.


## 8.8. Paediatric Cushing syndrome: a prospective, multisite, observational cohort study


Tatsi C, Kamilaris C, Keil M, Saidkhodjaeva L, Faucz FR, Chittiboina P, Stratakis CA



*Lancet Child Adolesc Health*. 2024; 8(1): 51-62.
https://pubmed.ncbi.nlm.nih.gov/38097317/


Brief Summary: This large patient cohort of children with endogenous Cushing syndrome describes their anthropometric, clinical, and biochemical characteristics, as well as their associated complications and outcomes. The findings inform diagnosis, treatment, and management.

Comment: Endogenous Cushing syndrome is rare in children and adolescents, and has variable manifestations (1-6). This prospective, multicenter cohort of 342 children and adolescents aged $\leq$18 years at diagnosis of Cushing syndrome collected clinical, biochemical, and imaging data until their latest appointment. 193 (56%) were female, 149 (44%) male, 261 (76%) had Cushing disease, 74 (22%) had adrenal-associated Cushing syndrome, and 7 (2%) had ectopic Cushing syndrome. Delay in diagnosis was median 2 years (IQR 1·0-3·0) after the first concerning sign or symptom. Patients with adrenal-associated Cushing syndrome were the youngest at diagnosis (median 10·4 years [IQR 7·4-13·6] vs 13·0 years [10·5-15·3] for Cushing disease vs 13·4 years [11·0-13·7] for ectopic Cushing syndrome). Body-mass index z-scores did not differ between

subtypes (1·90 [1·19-2·34] for adrenal-associated Cushing syndrome vs 2·18 [1·60-2·56] for Cushing disease vs 2·22 [1·42-2·35] for ectopic Cushing syndrome). Baseline biochemical screening for cortisol and ACTH at diagnosis showed overlapping results between subtypes, and especially between Cushing disease and ectopic Cushing syndrome. However, patients with ectopic Cushing syndrome had higher urinary free cortisol than thosse with adrenal-associated Cushing syndrome or Cushing disease.

Common complications of endogenous Cushing syndrome were decreased growth velocity, hypertension, hyperglycemia, elevated alanine transaminase, and dyslipidemia. Long-term recurrence was noted in at least 16/195 (8%) patients with Cushing disease. Recurrence was reported up to 8 years after surgery. This suggests that annual screening for recurrence should be performed in children for a minimum of 10 years after remission, and possibly longer, as recommended in adults.

In conclusion, this uniquely large patient cohort provides detailed information on the diagnostic evaluation of patients with this rare endocrine condition, as well as for screening for its complications. The prognosis of Cushing syndrome remains excellent if the diagnosis and management is performed by experienced specialists.

References


1. Hakami OA, Ahmed S, Karavitaki N. Epidemiology and mortality of Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab* 2021; 35: 101521.
2. Ragnarsson O, Olsson DS, Chantzichristos D, et al. The incidence of Cushing's disease: a nationwide Swedish study. *Pituitary* 2019; 22: 179–86.
3. Tatsi C, Stratakis CA. Pediatric Cushing syndrome; an overview. *Pediatr Endocrinol Rev* 2019; 17: 100–09.
4. Storr HL, Chan LF, Grossman AB, Savage MO. Paediatric Cushing's syndrome: epidemiology, investigation and therapeutic advances. *Trends Endocrinol Metab* 2007; 18: 167–74.
5. Magiakou MA, Mastorakos G, Oldfield EH, Gomez MT, Doppman JL, Cutler GB Jr, Nieman LK, Chrousos GP. Cushing's syndrome in children and adolescents. Presentation, diagnosis, and therapy. *N Engl J Med* 1994; 331: 629–36.
6. Karageorgiadis AS, Papadakis GZ, Biro J, Keil MF, Lyssikatos C, Quezado MM, Merino M, Schrump DS, Kebebew E, Patronas NJ, Hunter MK, Alwazeer MR, Karaviti LP, Balazs AE, Lodish MB, Stratakis CA. Ectopic adrenocorticotropic hormone and corticotropin-releasing hormone co-secreting tumors in children and adolescents causing cushing syndrome: a diagnostic dilemma and how to solve it. *J Clin Endocrinol Metab* 2015; 100: 141–48.


## 8.9. Consensus statement by the French Society of Endocrinology (SFE) and French Society of Pediatric Endocrinology & Diabetology (SFEDP) for the diagnosis of Cushing's syndrome: Genetics of Cushing's syndrome


Martinerie L, Bouligand J, North MO, Bertherat J, Assié G, Espiard S



*Ann Endocrinol (Paris)*. 2024; 85(4): 284-293.
https://pubmed.ncbi.nlm.nih.gov/38253221/



Brief Summary: This brief and concise Consensus statement by the French Society of Endocrinology (SFE) and French Society of Pediatric Endocrinology & Diabetology (SFEDP), describes a systematic review and provides recommendations for the use of genetic screening in Cushing's syndrome (CS).


Comment: The etiology of CS may involve both germline genetic alterations (detectable in leukocyte DNA) and somatic mutations (present only in tumor DNA). Two main pathways are altered in adrenal tumorigenesis: the catenin pathway and the cAMP/PKA pathway. Activation of the catenin pathway is involved mainly in adrenal tumors, particularly non-functional adenomas associated with moderate autonomous cortisol secretion and adrenocortical carcinomas (1, 2). Endogenous CS is due either to an ACTH-dependent source, most commonly pituitary adenomas (PAs), described as Cushing disease (CD), or less often ectopic CRH and/or ACTH secretion, or ACTH-independent (adrenal-related) hypercortisolemia (3).

Consideration of genetic evaluation follows identification of the type of CS. Germline mutations (typically in tumor-suppressor genes) in patients with ACTH-independent CS may explain more than half of the cases depending on the specific adrenal pathology (3). Tumor suppressor genes are inactivated according to Knudson's theory (4). An initial germline loss-of-function mutation (predisposition) is followed by a later somatic mutation (i.e., in the tumor), which may be a point mutation but is more often a gene deletion, resulting in "loss of heterozygosity" (4).

Given the rarity of genetic forms of Cushing's disease, there are currently insufficient data to recommend family genetic screening for isolated Cushing's disease, particularly in case of *AIP* mutations identified mainly in a sporadic context. For other genetic alterations responsible for CS, family genetic screening should be carried out according to the recommendations specific to each pathology. Thanks to recent technological advances, more and more genes are being identified as associated with Cushing's syndrome. Germline mutations are observed in around 50% of Cushing's syndrome of adrenal origin, depending on etiology, although this still concerns fewer than 5% of cases of Cushing's disease. Genetic testing is currently recommended in all cases of bilateral adrenal disease, as well as in all familial and/or pediatric forms of Cushing's syndrome of adrenal or pituitary origin. However, for a number of genes responsible for Cushing's syndrome, it is unclear whether taking these genetic alterations into account would modify the management of patients and their families.

References


1. Berthon A, Stratakis CA. From beta-catenin to ARM-repeat proteins in adrenocortical disorders. *Horm Metab Res.* 2014; 46(12): 889-96.
2. Espiard S, Ragazzon B, Bertherat J. Protein kinase A alterations in adrenocortical tumors. *Horm Metab Res.* 2014; 46(12): 869-75.
3. Tatsi C, Flippo C, Stratakis CA. Cushing syndrome: Old and new genes. *Best Pract Res Clin Endocrinol Metab.* 2020; 34(2): 101418.
4. Chernoff J. The two-hit theory hits 50. *Mol Biol Cell.* 2021; 32(22): rt1.


## 8.10. Management of phaeochromocytoma and paraganglioma in patients with germline SDHB pathogenic variants: an international expert consensus statement


Taïeb D, Nölting S, Perrier ND, Fassnacht M, Carrasquillo JA, Grossman AB, Clifton-Bligh R, Wanna GB, Schwam ZG, Amar L, Bourdeau I, Casey RT, Crona J, Deal CL, Del Rivero J, Duh QY, Eisenhofer G, Fojo T, Ghayee HK, Gimenez-Roqueplo AP, Gill AJ, Hicks R, Imperiale A, Jha A, Kerstens MN, de Krijger RR, Lacroix A, Lazurova I, Lin FI, Lussey-Lepoutre C, Maher ER, Mete O, Naruse M, Nilubol N, Robledo M, Sebag F, Shah NS, Tanabe A, Thompson GB, Timmers HJLM, Widimsky J, Young WJ Jr, Meuter L, Lenders JWM, Pacak K



*Nat Rev Endocrinol*. 2024; 20(3): 168-184.

https://pubmed.ncbi.nlm.nih.gov/38097671/


Brief Summary: The management of phaeochromocytomas and paragangliomas in patients with pathogenic variants of succinate dehydrogenase complex iron sulfur subunit B (*SDHD*) gene is complex. An international group of experts performed a critical review of the evidence to produce this consensus statement to assist clinical decision-making.

Comment: Pathogenic variants in succinate dehydrogenase complex iron sulfur subunit B (*SDHD*) gene are known causes of phaeochromocytomas and paragangliomas (PPGLs) (1). Their life-time disease penetrance is relatively high, and the recurrence rate for SDHB PPGLs is also high (2). Furthermore, these PPGLs are prone to aggressive behavior. Approximately 1/3 of patients develop metastases and have a predisposition to develop other tumors. Due to the complexity in clinical management of patients with *SDHB* PPGLs, the authors developed this consensus statement after reviewing the available evidence.

The authors comprised experts in PPGLs from a variety of countries, practice settings and disciplines. They reviewed published evidence and produced 29 graded recommendations on the clinical management of patients with an existing PPGL. The recommendations range from pre-operative work up to surveillance and different treatments. The authors strongly recommend that all major treatment and management decisions of patients with *SDHB* PPGLs should be carried out in an expert, interdisciplinary team setting. This approach facilitates personalized tailoring of patient management in specific clinical situations, including plans for individualized surveillance and follow-up. This consensus will facilitate standardized high-quality care for patients with PPGL due to *SDHB* pathogenic variants.

References


1. Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C; European-American Paraganglioma Study Group. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA.* 2004; 292(8): 943-51.
2. Assadipour Y, Sadowski SM, Alimchandani M, Quezado M, Steinberg SM, Nilubol N, Patel D, Prodanov T, Pacak K, Kebebew E. SDHB mutation status and tumor size but not tumor grade are important predictors of clinical outcome in pheochromocytoma and abdominal paraganglioma. *Surgery.* 2017; 161(1): 230-239.

## 8.11. Phase 3 Trial of Crinecerfont in Pediatric Congenital Adrenal Hyperplasia

Sarafoglou K, Kim MS, Lodish M, Felner EI, Martinerie L, Nokoff NJ, Clemente M, Fechner PY, Vogiatzi MG, Speiser PW, Auchus RJ, Rosales GBG, Roberts E, Jeha GS, Farber RH, Chan JL, Cahtalyst Pediatric Trial Investigators

*N Engl J Med*. 2024; 391(6):493-503.

https://pubmed.ncbi.nlm.nih.gov/38828945/

Brief Summary: This phase 3, multinational, randomized clinical trial (CAHtalyst, NCT04806451) in pediatric patients with CAH, evaluated the efficacy of crinecerfont to improve androgen control and enable GC dose reduction to a physiological range.

Comment: Congenital adrenal hyperplasia (CAH) comprises several rare autosomal recessive conditions resulting in disordered adrenal steroidogenesis. Pathogenic variants in the *CYP21A2* gene encoding steroid 21-hydroxylase, are responsible for ~95% of cases of CAH (1–4). Patients with classic CAH due to 21-hydroxylase deficiency have cortisol and often aldosterone deficiency from birth onwards (2). Glucocorticoids (GCs) are used for cortisol replacement therapy in patients with classical CAH. However, increased, supraphysiologic doses of GC are often needed to achieve adequate adrenal androgen reduction (1–4). Chronic supraphysiologic GC exposure results in multiple complications, such as decreased bone density, increased fracture risk, obesity, insulin resistance, diabetes mellitus, hyperlipidemia, hypertension, and psychological disturbances (1-6). One promising new strategy for reducing adrenal androgen overproduction through a GC-independent mechanism is corticotropin-releasing factor (CRF) type 1 receptor (CRF1) antagonism to reduce ACTH secretion, thus potentially allowing for physiological GC dosing (7). Crinecerfont is a novel oral CRF1 antagonist that reduced key hormone biomarkers in phase 2 studies in adults (NCT0352588627) and adolescents (NCT0404514528) with CAH.

This phase 3, multinational trial randomized (in 2:1 ratio) 103 children with classic CAH to receive crinecerfont or placebo for 28 weeks. A stable GC dose was maintained for 4 weeks, and the dose was then adjusted to a target of 8.0–10.0 mg/m$^2$/day of hydrocortisone dose equivalent, provided that the androstenedione concentration was controlled ($\leq$120% of the baseline concentration or within the reference range). Follow-up was high at 28 weeks (n = 100, 97%). At baseline, mean GC dose was 16.4 mg/m$^2$/day, and mean androstenedione concentration 431 ng/dL (15.0 nmol/L). At week 4, androstenedione was substantially reduced on crinecerfont (-197 ng/dL) but increased on placebo (+71 ng/dL) ($P < 0.001$); the mean androstenedione concentration prior to the morning GC dose was 208 ng/dL on crinecerfont, vs. 545 ng/dL on placebo. At week 28, mean GC dose had decreased (while androstenedione control was maintained) by 18.0% on crinecerfont but increased by 5.6% on placebo ($P < 0.001$).

These findings demonstrate the efficacy of crinecerfont to reduce elevated androstenedione concentrations in children with classical CAH. It also decreased GC doses from supraphysiologic to physiologic levels while maintaining androstenedione control.

References

1. Speiser PW, Arlt W, Auchus RJ, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2018; 103: 4043–88.
2. Claahsen-van der Grinten HL, Speiser PW, Ahmed SF, et al. Congenital adrenal hyperplasia-current insights in pathophysiology, diagnostics, and management. *Endocr Rev* 2022; 43: 91–159.
3. Mallappa A, Merke DP. Management challenges and therapeutic advances in congenital adrenal hyperplasia. *Nat Rev Endocrinol* 2022; 18: 337–52.
4. Merke DP, Auchus RJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *N Engl J Med* 2020; 383: 1248–61.
5. Turcu AF, Auchus RJ. Adrenal steroidogenesis and congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am* 2015; 44: 275–96.
6. Reisch N. Review of health problems in adult patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Exp Clin Endocrinol Diabetes* 2019; 127: 171–7.
7. Prete A, Auchus RJ, Ross RJ. Clinical advances in the pharmacotherapy of congenital adrenal hyperplasia. *Eur J Endocrinol* 2021; 186: R1–R14.

## 8.12. Ultradian hydrocortisone replacement alters neuronal processing, emotional ambiguity, affect and fatigue in adrenal insufficiency: the PULSES trial

Russell G, Kalafatakis K, Durant C, Marchant N, Thakrar J, Thirard R, King J, Bowles J, Upton T, Thai NJ, Brooks JCW, Wilson A, Phillips K, Ferguson S, Grabski M, Rogers CA, Lampros T, Wilson S, Harmer C, Munafo M, Lightman SL

*J Intern Med*. 2024; 295(1): 51-67.

https://pubmed.ncbi.nlm.nih.gov/37857352/

Brief Summary: This 6-week randomized, crossover, double blind, placebo-controlled feasibility trial assessed the effect of subcutaneous pump hydrocortisone on the quality of life, mood, functional neuroimaging, behavioral/cognitive responses, sleep and metabolism in adults with primary adrenal insufficiency (PAI) compared to standard therapy.

Comment: Adrenal glucocorticoid secretion is characterized by a complex diurnal variation, formed by changes in pulse amplitude of an underlying ultradian rhythm of short duration hormonal pulses (1). This trial (PULSES) administered usual dose hydrocortisone subcutaneously via a pump to provide safe circadian and ultradian cortisol replacement. Current usual replacement therapy with the standard, immediate-release hydrocortisone tablets cannot mimic physiologic cortisol secretion, lacks the pre-awakening cortisol surge and ultradian rhythmicity, and leads to unwanted post dose supraphysiologic cortisol peaks (2). Subcutaneous, pulsatile hydrocortisone administration altered both neural dynamics and behavioral responses related to emotional processing, visual stimulation and resting conditions, and improved physical and mental fatigue. It is well-known that HPA dysfunction is associated with depression, as well as poor sleep, well-being, mood and cognition (3).

Subjective mood responses were associated with altered functional neuroimaging responses to external stimulation, with the different temporal patterns of plasma cortisol, impacting brain areas important for emotional encoding, such as the amygdala, insula and frontal cortical regions. Physiologic stress response is tightly connected to that of the salience network, with stress hormones likely potentiating the intra-network functional connectivity, all adaptive changes favoring proper decisions and survival (4). Irrespective of treatment, participants had significant sleep disturbances, and treatment modality had no effect on sleep quality, although improved behavior post awakening, and ease of awakening was seen on pulsatile therapy. Studies have shown variable effects on slow wave sleep and reduced REM latency in PAI, all of which potentially impact cognition and emotion, through alterations in sleep fatigue and REM sleep (5). Pulsatility also improved positive mood, while no effect was observed on either working memory or metabolic parameters over the 6-week trial. It has been suggested that glucocorticoid pulsatility constitutes a regulatory factor for processes involved in cognitive psychophysiology, including (i) self-perceived domains of well-being, (ii) daily mood oscillations, and (iii) resting state neural dynamics, independently and in the context of mood regulation (1).

There are several novel approaches to mimic circadian rhythmicity in hydrocortisone treatment, including once-daily and modified-release oral preparations. However, those also do not address the normal pulsatility of cortisol secretion. Future studies in PAI patients are needed with long duration of treatment modalities, to better characterize their neuropsychological effects. This should hopefully provide the evidence needed to improve glucocorticoid treatment regimens, reduce the morbidity of current replacement therapy and reduce the long-term neuropsychiatric comorbidities in these patients.

References


1. Kalafatakis K, Russell GM, Ferguson SG, Grabski M, Harmer CJ, Munafo MR, et al. Glucocorticoid ultradian rhythmicity differentially regulates mood and resting state networks in the human brain: A randomised controlled clinical trial. *Psychoneuroendocrinology*. 2021; 124: 105096.
2. Russell G, Lightman S. The human stress response. *Nat Rev Endocrinol.* 2019; 15(9): 525-34.
3. Pace TW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav Immun.* 2007; 21(1): 9-19.
4. Paltoglou G, Stefanaki C, Chrousos GP. Functional MRI Techniques Suggesting that the Stress System Interacts with Three Large Scale Core Brain Networks to Help Coordinate the Adaptive Response: A Systematic Review. *Curr Neuropharmacol.* 2024; 22(5): 976-89.
5. Henry M, Ross IL, Thomas KGF. Reduced Slow-Wave Sleep and Altered Diurnal Cortisol Rhythms in Patients with Addison's Disease. *Eur J Endocrinol.* 2018; 179(5): 319-30.


New Hope

## 8.13. The role of interferon-γ in autoimmune polyendocrine syndrome Type 1


Oikonomou V, Smith G, Constantine GM, Schmitt MM, Ferré EMN, Alejo JC, Riley D, Kumar D, Dos Santos Dias L, Pechacek J, Hadjiyannis Y, Webb T, Seifert BA, Ghosh R, Walkiewicz M, Martin D, Besnard M, Snarr BD, Deljookorani S, Lee CR, DiMaggio T, Barber P, Rosen LB, Cheng A, Rastegar A, de Jesus AA, Stoddard J, Kuehn HS, Break TJ, Kong HH, Castelo-Soccio L, Colton B, Warner BM, Kleiner DE, Quezado MM, Davis JL, Fennelly KP, Olivier KN, Rosenzweig SD, Suffredini AF, Anderson MS, Swidergall M, Guillonneau C, Notarangelo LD, Goldbach-Mansky R, Neth O, Monserrat-Garcia MT, Valverde-Fernandez J,

Lucena JM, Gomez-Gila AL, Garcia Rojas A, Seppänen MRJ, Lohi J, Hero M, Laakso S, Klemetti P, Lundberg V, Ekwall O, Olbrich P, Winer KK, Afzali B, Moutsopoulos NM, Holland SM, Heller T, Pittaluga S, Lionakis MS
*N Engl J Med*. 2024; 390(20): 1873-1884.
https://pubmed.ncbi.nlm.nih.gov/38810185/

Brief Summary: This study suggests that excessive interferon-γ–mediated responses have a pathogenic role in APS-1 and provides the foundation for therapies that affect interferon-γ–mediated disease.

Commentary: Autoimmune polyendocrine syndrome type 1 (APS-1), also known as autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (APECED), is an autosomal recessive multiorgan syndrome caused by loss-of-function variants in *AIRE*, the gene encoding autoimmune regulator (1-3). In APS-1, self-reactive T cells escape thymic negative selection, infiltrate organs, and drive autoimmune injury. APS-1 manifests in childhood with a characteristic triad of chronic mucocutaneous candidiasis, adrenal insufficiency, and hypoparathyroidism, alongside numerous other endocrine and nonendocrine diseases (4-6). Mortality can exceed 30% despite supportive care (7). Although progress has been made in the treatment of certain tissue-specific autoimmune manifestations, no therapy targets the multiorgan nature of APS-1.

These authors performed exploratory studies in patients with APS-1 and in Aire−/− mice to study mechanisms of T-cell–mediated tissue injury and to test therapeutic strategies. Their findings suggested that APS-1 is an interferon-γ– mediated disease. Patients with APS-1 had enhanced interferon-γ responses in blood and in all examined autoimmunity-affected tissues. Aire-/- mice had selectively increased interferon-γ production by T cells and enhanced interferon-γ, phosphorylated signal transducer and activator of transcription 1 (pSTAT1), and CXCL9 signals in multiple organs. Ifng ablation or ruxolitinib-induced JAK-STAT blockade in Aire-/- mice normalized interferon-γ responses and averted T-cell infiltration and damage in organs. They then treated 5 patients with APS-1 with ruxolitinib, a Food and Drug Administration (FDA)–approved Janus kinase (JAK) 1 and 2 inhibitor (15-17). Ruxolitinib led to decreased levels of T-cell-derived interferon-γ, normalized interferon-γ and CXCL9 levels, and remission of alopecia, oral candidiasis, nail dystrophy, gastritis, enteritis, arthritis, Sjögren's-like syndrome, urticaria, and thyroiditis. No serious adverse effects were observed.

These findings indicate that APS-1 is characterized by excessive, multiorgan interferon-γ-mediated responses. JAK inhibition with ruxolitinib in five patients showed promising results.

These data provide new insights into the mechanism and pathophysiological basis of APS-1 and uncover important targets for therapeutic intervention.

References


1. Oftedal BE, Hellesen A, Erichsen MM, et al. Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune diseases. *Immunity* 2015; 42: 1185-96.
2. Ricotta EE, Ferré EMN, Schmitt MM, DiMaggio T, Lionakis MS. Prevalence of APECED-like clinical disease in an electronic health record database, USA. *J Clin Immunol* 2022; 42: 904-6.
3. Constantine GM, Lionakis MS. Lessons from primary immunodeficiencies: autoimmune regulator and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Immunol Rev* 2019; 287: 103-20.
4. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasisectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 1990; 322: 1829-36.
5. Bruserud Ø, Oftedal BE, Landegren N, et al. A longitudinal follow-up of autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab* 2016; 101: 2975-83.
6. Ferre EMN, Rose SR, Rosenzweig SD, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* 2016; 1(13): e88782.
7. Borchers J, Pukkala E, Mäkitie O, Laakso S. Patients with APECED have increased early mortality due to endocrine causes, malignancies and infections. *J Clin Endocrinol Metab* 2020; 105(6): e2207-e2213.
8. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Engl J Med* 2015; 372: 426-35.
9. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med* 2012; 366: 799-807.
10. Zeiser R, Polverelli N, Ram R, et al. Ruxolitinib for glucocorticoid-refractory chronic graft-versus-host disease. *N Engl J Med* 2021; 385: 228-38.


## 8.14. Circulating non-coding RNA biomarkers of endocrine tumours

Butz H, Patócs A, Igaz P
*Nat Rev Endocrinol*. 2024;
doi: 10.1038/s41574-024-01005-8. PMID: 38886617.
https://pubmed.ncbi.nlm.nih.gov/38886617/

Brief Summary: This review provides a comprehensive summary of the current research, pathophysiologic mechanisms and methodological factors regarding the utility of ncRNA as biomarkers for endocrine tumors.

Comment: Clinical management of endocrine tumors has several challenges, including preoperative diagnosis regarding malignancy, monitoring of treatment and patient follow-up. The currently available biomarkers for endocrine tumors have important limitations (1). Non-coding RNAs (ncRNAs) exist in various forms and exert various regulatory roles (2). Circulating ncRNAs show promising utility as biomarkers of malignancy, prognosis and follow-up in several endocrine tumors, because they act beyond their traditional role as intracellular regulators to being systemic regulators of gene expression (3). Tumor cells represent a significant source of circulating ncRNA.

In this review, the authors summarize and describe key studies on circulating ncRNAs in endocrine tumors. Moreover, they also review the methodological, biological and technical aspects of the use of ncRNAs as clinical biomarkers.

References

1. Herrera-Martinez AD.et al. Neuroendocrine neoplasms: current and potential diagnostic, predictive and prognostic markers. *Endocr. Relat. Cancer* 2019; 26: R157–R179.
2. Anfossi S, Babayan A, Pantel K, Calin GA. Clinical utility of circulating non-coding RNAs — an update. *Nat Rev Clin Oncol* 2018; 15: 541–563.
3. Cortez MA. et al. MicroRNAs in body fluids — the mix of hormones and biomarkers. *Nat Rev Clin Oncol* 2011; 8: 467–477.

## 8.15. Metastatic pheochromocytoma and paraganglioma: somatostatin receptor 2 expression, genetics, and therapeutic responses

Fischer A, Kloos S, Maccio U, Friemel J, Remde H, Fassnacht M, Pamporaki C, Eisenhofer G, Timmers HJLM, Robledo M, Fliedner SMJ, Wang K, Maurer J, Reul A, Zitzmann K, Bechmann N, Žygienė G, Richter S, Hantel C, Vetter D, Lehmann K, Mohr H, Pellegata NS, Ullrich M, Pietzsch J, Ziegler CG, Bornstein SR, Kroiss M, Reincke M, Pacak K, Grossman AB, Beuschlein F, Nölting S

*J Clin Endocrinol Metab*. 2023; 108(10): 2676-2685.
https://pubmed.ncbi.nlm.nih.gov/36946182/

Brief Summary: This study explored the relationship between Somatostatin Receptor 2 (SSTR2) immunoreactivity and succinate dehydrogenase complex iron sulfur subunit B (SDHB) immunoreactivity, mutational status, and clinical behavior of paragangliomas (PPGLs), and evaluated SSTR-based therapies in metastatic PPGLs. The findings highlight SSTR2 expression as a novel biomarker for metastatic behavior in PCC, PGL and SDHB/ SDHx mutations. They also suggest that SSTR-based therapies may provide superior therapeutic outcomes in metastatic PPGLs with a higher disease control rate.

Comment: Pheochromocytomas (PCC) and paragangliomas (PPGLs) caused by pathogenic mutations in succinate dehydrogenase subunit B (*SDHB*) gene are rare inheritable neuroendocrine tumors with a high propensity for metastasis, making their management particularly challenging (1). Therefore, identifying biomarkers of potential malignant risk is of great clinical importance.

This retrospective study focuses on the expression of somatostatin receptor 2 (SSTR2), which is present in both non-metastatic and metastatic PPGLs, as a potential key biomarker in the diagnosis and treatment of neuroendocrine tumors, as it is the target for imaging (2-4). The study's primary objectives were to evaluate the correlation between SSTR2 immunoreactivity, SDHB immunoreactivity, mutational status and the clinical phenotype of PPGLs. Additionally, the study assessed the efficacy of SSTR based therapies, including peptide receptor radionuclide therapy (PRRT) and cold SSTR2 analogues, in treating metastatic PPGLs.

SSTR2 was positive in 50% of patients (n = 101), although with considerable variability in intensity. SSTR2 positivity was not linked to tumor location, size, or a higher proliferation index (Ki-67 > 3%). However, it was associated with SDHB and SDHx related PPGLs, with the highest intensity observed in SDHB related PPGLs, and with metastatic disease independent of *SDHB/SDHx* mutation status. Furthermore, the disease control rate in metastatic PPGLs was 67% with first-line SSTR-based radionuclide therapy (n = 22, n = 11SDHx) and 100% with first line "cold" somatostatin analogues (n = 6, n = 3 SDHx).
In conclusion, this study offers valuable insights into the potential of SSTR2 expression particularly as a novel

biomarker for diagnosing metastatic behavior in *PCC, PGL* and *SDHB/ SDHx* mutations. It also suggests that SSTR-based therapies may provide superior therapeutic outcomes in metastatic PPGLs with a higher disease control rate.


References

1. Crona J, Lamarca A, Ghosal S, Welin S, Skogseid B, Pacak K. Genotype-phenotype correlations in pheochromocytoma and paraganglioma: a systematic review and individual patient meta-analysis. *Endocr Relat Cancer.* 2019; 26(5): 539–550.
2. Reubi J, Waser B, Schaer JC, Laissue JA. Somatostatin receptor sst1–sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med.* 2001; 28(7): 836-846.
3. Taïeb D, Jha A, Treglia G, Pacak K. Molecular imaging and radionuclide therapy of pheochromocytoma and paraganglioma in the era of genomic characterization of disease subgroups. *Endocr Relat Cancer.* 2019; 26(11): R627-R652.
4. Janssen I, Blanchet EM, Adams K, et al. Superiority of [68Ga]-DOTATATE PET/CT to other functional imaging modalities in the localization of SDHB -associated metastatic pheochromocytoma and paraganglioma. *Clin Cancer Res.* 2015; 21(17): 3888-3895.


## New Genes

### 8.16. Single-exon deletions of ZNRF3 exon 2 cause congenital adrenal hypoplasia


Amano N, Narumi S, Aizu K, Miyazawa M, Okamura K, Ohashi H, Katsumata N, Ishii T, Hasegawa T

*J Clin Endocrinol Metab*. 2024; 109(3): 641-648.

https://pubmed.ncbi.nlm.nih.gov/37878959/


Brief Summary: This study identifies a novel cause for congenital adrenal hypoplasia and provides evidence that Wnt/β-catenin signaling plays an important role in the development of human adrenal cortex.

Comment: Primary adrenal insufficiency (PAI) is a life-threatening condition characterized by the inability of the adrenal cortex to produce sufficient glucocorticoids and/or mineralocorticoids. The major cause of childhood-onset PAI is congenital adrenal hyperplasia, such as 21-hydroxylase deficiency (1), however, a small subset of patients lack a known cause for their disorder.

These authors enrolled patients with PAI (n = 9) of genetically unknown cause. They used array comparative genomic hybridization to identify a heterozygous deletion of exon 2 in the zinc and ring finger 3 (*ZNRF3*) gene. Further studies showed that this deletion results in the expression of a 42-amino acid shorter protein. Loss of this exon impaired interaction between ZNRF3 and RSPO1 on 3D modelling, indicating a potential defect in the RSPO1-dependent activation of the Wnt/β-catenin pathway. In a cell-based functional assay, RSPO1-dependent activation of the Wnt/β-catenin pathway was indeed impaired, indicating the importance of ZNRF3 in the development of the adrenal cortex. These findings extend the list of genetic causes of PAI.


Reference

1. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev.* 2000; 21(3): 245-291.


## New Paradigms

### 8.17. Increased resting-state functional connectivity in patients with autoimmune addison disease


Van't Westeinde A, Padilla N, Fletcher-Sandersjöö S, Kämpe O, Bensing S, Lajic S

*J Clin Endocrinol Metab*. 2024; 109(3): 701-710.

https://pubmed.ncbi.nlm.nih.gov/37820745/


Brief Summary: This case-control study compared resting-state functional connectivity (rs-fc) of the brain between patients with autoimmune Addison's disease (AAD) and healthy controls. The results suggest that AAD affects the baseline functional organization of the brain and that current treatment strategies of AAD may be a risk factor.

Comment: Autoimmune Addison disease (AAD) is a form of primary adrenal insufficiency (PAI), in which autoimmune adrenal cortex destruction results in chronic glucocorticoid (GC) and mineralocorticoid (MC) deficiency that require life-long replacement treatment at the lowest dose possible to prevent negative side effects from cortisol overdosing (1). These side-effects offer scientists a pathophysiologic insight into the widespread effects of cortisol, which affect many physiologic systems. In the CNS, brain widely expresses both GC and MC receptors, and cortisol can affect both the anatomical structure and the functional activity and connectivity (FC) of the brain on a short-term and long-term basis (2). The relation between cortisol-related metabolic changes and direct effects on neuronal excitability and changes in rs connectivity signal are unclear. Neurobiology of chronic insomnia is linked to hypothalamic-pituitary-adrenal-axis dysregulation, and in particular, alterations in circadian and ultradian cortisol rhythmicity, which affect many cognitive and affective processes, including long-term memory, working memory, and emotional regulation, particularly in response to stressful situations (3). Stress system activation is tightly connected to Salience (SN), Default Mode (DMN) and Central Executive (CEN) networks' activation, with stress hormones likely potentiating the intra-network FC of the latter, attenuating that of the DMN, and causing a biphasic suppression-to-activation response of the CEN, all adaptive changes favoring proper decisions and survival (4).

In this study, patients with AAD had increased rs-fc within 3 major networks, namely the orbitofrontal cortex (OFC), the posterior DMN and the medial visual network, while being on a relatively higher GC replacement dose was associated with stronger rs-fc in a small part of the OFC network. These changes are associated with regions (particularly the OFC) that show the strongest reduction in volume in patients with AAD (5). In patients with CAH, stronger rs-fc in the precuneus compared to healthy controls has been observed, while in patients with Cushing disease increases in rs-fc in several networks, including the posterior cingulate cortex/precuneus and the prefrontal cortex, the subgenual ACC-DMN and the medial temporal (right parahippocampal gyrus) and medial prefrontal cortex have been described, even following remission (6-8).

The networks that showed group differences were the OFC and the DMN. The OFC (or ventromedial prefrontal cortex) is mostly known for containing a high density of GC receptors, and is involved in many higher- order cognitive processes, such as motivation and emotion regulation, processing reward-related information needed for emotional and social behavior, but also shaping autonomic and endocrine responses, including stress (9-11). The DMN is involved in a wide variety of tasks, while it is typically deactivated during most stimulus-driven cognitive tasks – and during acute stress (4). It is hypothesized that DMN provides the functional infrastructure for integrating past, present and future events related to the self (12).

In conclusion, patients with AAD have stronger rs-fc in several brain networks, partly correlating with GC replacement dose. Further studies are needed to determine if these changes predispose individuals to problems with cognition and mood later in life or if they are part of a compensation mechanism. Both psychologic and brain health need to be considered when optimizing replacement therapy, while GC replacement therapy that mimics normal cortisol secretion should be preferred.

References


1. Husebye ES, Pearce SH, Krone NP, Kampe O. Adrenal insufficiency. *Lancet.* 2021; 397(10274): 613-29.
2. Joels M. Corticosteroids and the brain. J Endocrinol. 2018; 238(3): R121-R30.
3. Vargas I, Vgontzas AN, Abelson JL, Faghih RT, Morales KH, Perlis ML. Altered ultradian cortisol rhythmicity as a potential neurobiologic substrate for chronic insomnia. *Sleep Med Rev.* 2018; 41: 234-43.
4. Paltoglou G, Stefanaki C, Chrousos GP. Functional MRI Techniques Suggesting that the Stress System Interacts with Three Large Scale Core Brain Networks to Help Coordinate the Adaptive Response: A Systematic Review. *Curr Neuropharmacol.* 2024; 22(5): 976-89.
5. Van't Westeinde A, Padilla N, Siqueiros Sanchez M, Fletcher-Sandersjoo S, Kampe O, Bensing S, et al. Brain structure in autoimmune Addison's disease. *Cereb Cortex.* 2023; 33(8): 4915-26.
6. Messina V, Van't Westeinde A, Padilla N, Lajic S. Changes in resting-state functional connectivity in patients with congenital adrenal hyperplasia. *Neuroimage Clin.* 2022; 35: 103081.
7. Jiang H, He NY, Sun YH, Jian FF, Bian LG, Shen JK, et al. Altered spontaneous brain activity in Cushing's disease: a resting-state functional MRI study. *Clin Endocrinol (Oxf).* 2017; 86(3): 367-76.
8. Stomby A, Salami A, Dahlqvist P, Evang JA, Ryberg M, Bollerslev J, et al. Elevated resting-state connectivity in the medial temporal lobe and the prefrontal cortex among patients with Cushing's syndrome in remission. *Eur J Endocrinol.* 2019; 180(5): 329-38.
9. Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Prog Neurobiol.* 2004; 72(5): 341-72.
10. Maier SU, Makwana AB, Hare TA. Acute Stress Impairs Self-Control in Goal-Directed Choice by Altering Multiple Functional Connections within the Brain's Decision Circuits. *Neuron.* 2015; 87(3): 621-31.
11. Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci.* 1993; 13(9): 3839-47.
12. Buckner RL, Carroll DC. Self-projection and the brain. *Trends Cogn Sci.* 2007; 11(2): 49-57.

## 8.18. The association of accelerated early growth, timing of puberty, and metabolic consequences in children

Jee YH, Jumani S, Mericq V

*J Clin Endocrinol Metab*. 2023; 108(9): e663-e670.
https://pubmed.ncbi.nlm.nih.gov/37029976/

Brief Summary: The mechanism by which suboptimal intrauterine growth and low birth weight for gestational age may lead to postnatal metabolic risks has not been well established. This narrative review summarizes recent studies on the association between early infant growth, timing of puberty, and metabolic risks, thus expanding our knowledge of the underlying pathophysiology.

Comment: Early compensatory infantile growth in children born small for gestational age (SGA), i.e. involving abnormal intrauterine or prenatal growth and/or early accelerated postnatal growth in children with premature adrenarche (PA), is associated with an increased propensity for cardiometabolic risk factors or metabolic syndrome phenotype later in life (1). Changes in metabolic programming by leptin signaling, epigenetic reprogramming of mesenchymal stem cells or alteration of DNA methylation have been proposed, while genetic or maternal factors that are associated with either the above described abnormal growth patterns or the appearance of metabolic syndrome in adulthood have also been studied (2-4). In addition, if malnutrition in prenatal life is followed by abundant nutrition after birth, it may induce metabolic programming of the fetal organs that predisposes them to insulin resistance and metabolic syndrome ("thrifty phenotype hypothesis") (5). Further research is needed to establish the best approach to prevent the metabolic programming favoring metabolic risks during the nutritional replenish phase.

The common initial pathophysiologic pathway seems to involve early adrenal maturation (6). Adrenarche begins in early childhood, due to maturation of the adrenal cortex zona reticularis, with a concomitant gradual increase in adrenal androgen production (7). The most sensitive biochemical marker for adrenarche is sulfated dehydroepiandrosterone (DHEA-S) concentrations (8). DHEA-S is produced in the fetal adrenal gland, its concentrations are high at birth but then they rapidly decline during infancy and rise again gradually at onset of adrenarche, reaching peak level in the second decade of life (6). This DHEA-S elevation is also a marker of PA that can clinically be defined by the appearance of symptoms (adult body odor, oily skin, acne, pubic and axillary hair) at an earlier than the expected age (6). Of note, children born SGA, especially after catch-up growth, have elevated DHEA-S concentrations between 6 and 8 years of age and are more prone to develop PA (9).

Children born SGA are at increased risk of early puberty, associated with early maturation of the zona reticularis and increased DHEA-S following early catch-up growth (10). This hypothesis has been extensively studied and it is possibly mediated by DHEA-S and other adrenal androgens (11, 12). In addition, investigations using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) have broadened the traditional androgen repertoire to include other androgens with higher binding capacity to the androgen receptor (13).

Earlier age of pubertal onset is associated with high BMI in childhood, while PA may be independently associated with increased cardiometabolic risk (14). The hypothesis of this 3-way reciprocal association potentially links increased body weight with early onset adrenarche or/and puberty with cardiometabolic risk. The pathogenetic starting point (primum movens) remains to be elucidated, with novel studies and approaches that include detailed CNS functional imaging (functional MRI) (15). Underlying genetic variants may explain the association between adrenal androgens and metabolic risks.

In summary, studies agree that pubertal timing is a risk factor, which independently influences several metabolic disease-related traits, including the degree of obesity, serum lipids, and insulin, both in adult females and males. The observed connection between pubertal timing and adult metabolic outcomes implies that mechanisms that advance puberty may also contribute to adult metabolic disorders. As adult metabolic disease risk is shaped over the life course, understanding this link and the critical developmental events may highlight important pathogenic mechanisms. They may also inform more specific individualised prevention strategies. Future studies, including birth history, early infantile growth, various adrenal androgens, and timing of puberty are required to assess metabolic disease risks in addition to suitable animal models to study the sequence of SGA, adrenal maturation and metabolic disorders.

References

1. Nordman H, Jaaskelainen J, Voutilainen R. Birth Size as a Determinant of Cardiometabolic Risk Factors in Children. *Horm Res Paediatr*. 2020; 93(3): 144-53.
2. Lewandowski KC, Biesiada L, Grzesiak M, Sakowicz A. C-Peptide and leptin system in dichorionic, small and appropriate for gestational age twins-possible link to metabolic programming? *Nutr Diabetes.* 2020; 10(1): 29.
3. Peeters S, Declerck K, Thomas M, Boudin E, Beckers D, Chivu O, et al. DNA Methylation Profiling and Genomic Analysis in 20 Children with Short Stature Who Were Born Small for Gestational Age. *J Clin Endocrinol Metab.* 2020; 105(12).
4. Beaumont RN, Kotecha SJ, Wood AR, Knight BA, Sebert S, McCarthy MI, et al. Common maternal and fetal genetic variants show expected polygenic effects on risk of small- or large-for-gestational-age (SGA or LGA), except in the smallest 3% of babies. *PLoS Genet*. 2020; 16(12): e1009191.
5. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull.* 2001; 60: 5-20.
6. Rosenfield RL. Normal and Premature Adrenarche. *Endocr Rev*. 2021; 42(6): 783-814.
7. Palmert MR, Hayden DL, Mansfield MJ, Crigler JF, Jr., Crowley WF, Jr., Chandler DW, et al. The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. *J Clin Endocrinol Metab.* 2001; 86(9): 4536-42.
8. Rege J, Turcu AF, Kasa-Vubu JZ, Lerario AM, Auchus GC, Auchus RJ, et al. 11-Ketotestosterone Is the Dominant Circulating Bioactive Androgen During Normal and Premature Adrenarche. *J Clin Endocrinol Metab.* 2018; 103(12): 4589-98.
9. Ibanez L, Lopez-Bermejo A, Diaz M, Suarez L, de Zegher F. Low-birth weight children develop lower sex hormone binding globulin and higher dehydroepiandrosterone sulfate levels and aggravate their visceral adiposity and hypoadiponectinemia between six and eight years of age. *J Clin Endocrinol Metab.* 2009; 94(10): 3696-9.
10. Iniguez G, Ong K, Bazaes R, Avila A, Salazar T, Dunger D, et al. Longitudinal changes in insulin-like growth factor-I, insulin sensitivity, and secretion from birth to age three years in small-for-gestational-age children. *J Clin Endocrinol Metab.* 2006; 91(11): 4645-9.
11. Mantyselka A, Jaaskelainen J, Eloranta AM, Vaisto J, Voutilainen R, Ong K, et al. Associations of lifestyle factors with serum dehydroepiandrosterone sulphate and insulin-like growth factor-1 concentration in prepubertal children. *Clin Endocrinol (Oxf).* 2018; 88(2): 234-42.
12. Liimatta J, Utriainen P, Laitinen T, Voutilainen R, Jaaskelainen J. Cardiometabolic Risk Profile Among Young Adult Females with a History of Premature Adrenarche. *J Endocr Soc.* 2019; 3(10): 1771-83.
13. Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC, et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *J Clin Endocrinol Metab.* 2013; 98(3): 1182-8.
14. Widen E, Silventoinen K, Sovio U, Ripatti S, Cousminer DL, Hartikainen AL, et al. Pubertal timing and growth influences cardiometabolic risk factors in adult males and females. *Diabetes Care.* 2012; 35(4): 850-6.
15. Tzounakou AM, Stathori G, Paltoglou G, Valsamakis G, Mastorakos G, Vlahos NF, Charmandari E. Childhood Obesity, Hypothalamic Inflammation, and the Onset of Puberty: A Narrative Review. *Nutrients.* 2024;16(11).

## Food for Thought

## 8.19. Prenatal androgen exposure and sex-typical play behaviour: a meta-analysis of classic congenital adrenal hyperplasia studies

Kung KTF, Louie K, Spencer D, Hines M

*Neurosci Biobehav Rev.* 2024; 159: 105616.
https://pubmed.ncbi.nlm.nih.gov/38447820/

Brief Summary: This meta-analysis of studies of classical CAH indicates that prenatal androgen exposure masculinizes and defeminizes play behavior in humans.

Comment: Sex differences in juvenile-play behavior have been documented across a number of animal species, including non-human primates and in humans. It is proposed that juvenile play allows the developing individual to practice skills that are important for survival and adult social roles. In humans, average sex differences in play appear early in life and persist throughout childhood. By 12 months of age, sex-typical toy preferences emerge and by 2-3 years, boys and girls seem to prefer same-sex playmates and sex-segregated play. By 3 years, sex differences in play become more apparent; boys engage in more rough-and tumble and competitive play, whilst girls tend to engage in more verbal and nurturant play. These sex differences persist across the entire childhood (1). Compared to other human behavioral sex-differences, the sex-typical toy play behavior has a large effect size. Sex differences in play behavior are dependent on prenatal and neonatal androgen exposure during critical periods of early fetal and neonatal life, when androgen concentrations in males exceed those in females. There is a linear and positive relationship between androgen exposure and level of male-typical play behavior (2, 3). Areas in the brain that are re-organized by androgen exposure and that might affect the behavioral differences are the sexually dimorphic nucleus of the pre-optic area in rats and rams, the Onuf's nucleus in rhesus macaques, and the amygdala, all of which are larger in males than females.

This study is a review and meta-analysis of studies investigating sex-typical play behaviour, and the effects of prenatal androgen exposure using congenital adrenal hyperplasia (CAH) as a model system. The meta-analysis

included 20 independent samples, in total 517 females with CAH, 556 control females, 176 males with CAH, and 259 control males. Consistently across all 7 male-typical and female-typical play outcomes (male/female play behavior, male/female toy preferences, male playmate preferences, male/female overall play behavior), there were large differences between control males and control females (Hedges' gs = 0.83-2.78), as well as between females with CAH and control females (Hedges' gs = 0.95-1.08). However, differences between males with CAH and control males were mostly negligible and were non-significant for 6 of the 7 outcomes (Hedges' gs = 0.04-0.27). Prader scores or CYP21A2 genotypes were not included as moderators since very few studies reported these parameters.

In summary, the results suggest that females with CAH are between control females and control males when it comes to toy-play behaviors. This supports the notion that prenatal androgen concentrations influence sex-typical play behavior so that androgen exposure masculinizes and defeminizes play behaviour in humans. There was no effect of participant age or publication year, which are crude proxies of socio-cognitive or cultural influences.

## References


1. Davis JTM, Hines M. How large are gender differences in toy preferences? A systematic review and meta-analysis of toy preference research. *Arch. Sex. Behav.* 2020; 49: 373-394.
2. Hines M. Brain gender. Oxford University Press, New York, NY. 20024
3. Hines M. Human gender development. *Neurosci. Biobehav. Rev.* 2020; 118: 89-96.

# 9. Oncology and Chronic Disease

Carla Bizzarri, Sara Ciccone, Stefania Pedicelli
Unit of Endocrinology and Diabetes, Bambino Gesu' Children's Hospital, IRCCS, Rome, Italy
carla.bizzarri@opbg.net

## Introduction

Survival rates for childhood cancers have steadily improved over the past decades. These changes have shifted the concerns of parents and patients. While survival was previously the primary concern, late effects of cancer treatment are now becoming a major health issue. With a 5-year survival rate exceeding 85% for all cancers combined in this age group, many young survivors will live long enough to have biological children, but they may have concerns about the potential impact of cancer treatment on their reproductive outcomes. Large epidemiological studies have reported a significant reduction in fertility in both sexes. Recent studies conducted on adult cancer patients have shown that cancer itself can damage germ cells, even before any treatment. Recent data confirm that cancer survivors perceive an increased risk of infertility but often struggle to accurately estimate their specific risk. Infertility risk counseling can help to reduce the discrepancy between perception and actual risk, decrease psychological distress, and properly inform family planning decisions.

## Fertility Issues

### 9.1. Premature ovarian insufficiency and chance of pregnancy after childhood cancer: a population-based study (the Fex-Can study)

Haavisto A, Wettergren L, Lampic C, Lähteenmäki PM, Jahnukainen K
anu.haavisto@helsinki.fi,
*Int J Cancer.* 2023 Aug 1;153(3):644-653.
doi: 10.1002/ijc.34541. PMID: 37078589

Brief Summary: This single centre cross-sectional study used a self-reported questionnaire to evaluate ovarian function in 1333 female young adult childhood cancer survivors (CCS).

The authors used two different indicators of primary ovarian insufficiency (POI): induced puberty, reported in 5.3% and estrogen replacement therapy (ERT) at assessment, reported in 9.3%. Induced puberty was correlated with more aggressive cancer treatments, in particular the use of abdominal irradiation. A cut-off of 36 Gy showed 100% sensitivity in predicting the need for puberty induction, while a dose of 24 Gy had 100% specificity. ERT was more common in patients who had undergone hematopoietic stem cell transplantation (HSCT) or abdominal irradiation. ERT in the context of multiple hormone deficiencies was more common in patients with high-grade CNS neoplasms (9.9%), patients receiving CNS radiotherapy (15.7%) or HSCT (8.3%).

One of the major detrimental late effects of cancer treatment in women is impaired ovarian function. Premature ovarian insufficiency (POI) includes acute ovarian failure (AOF), as absence of menarche or cessation of menstruation, and premature menopause. The frequency of POI in CCS is estimated to be 11-13%, with a consequently reduced incidence of pregnancy (28-31%), compared to the general population. This study confirms that HSCT is the strongest predictor of POI, need of fertility treatments and reduced chance of pregnancy; chemotherapy and pelvic or CNS irradiation are also risk factors. The importance of psychological and social factors is also highlighted. The use of a self-reported questionnaire is the main limitation of this study. More detailed assessments using laboratory data to diagnose POI are needed to provide more precise information to clinicians and patients.

References

1. Chemaitilly W, Li Z, Krasin MJ, et al. Premature ovarian insufficiency in childhood cancer survivors: a report from the St. Jude lifetime cohort. *J Clin Endocrinol Metab.* 2017;102(7):2242-2250.
2. Netterlid A, Mörse H, Giwercman A, et al. Premature ovarian failure after childhood cancer and risk of metabolic syndrome: a crosssectional analysis. *Eur J Endocrinol.* 2021;185(1):67-75.

## 9.2. Effects of radiation therapy on the female reproductive tract in childhood cancer survivors: a PENTEC comprehensive review

Hill-Kayser C, Yorke E, Jackson A, Gracia C, Ginsberg J, Keene K, Ronckers C, Metzger ML, Li Z, Constine LS, Hua CH
Christine.Hill@pennmedicine.upenn.edu,
*Int J Radiat Oncol Biol Phys.* 2024 Jun 1;119(2):588-609.
doi: 10.1016/j.ijrobp.2023.08.013. PMID: 37804257

Brief Summary: This paper reviewed the toxicity of radiation therapy (RT) on ovarian, uterine and vaginal tissue in childhood cancer survivors (CCSs), using data published from 1970 to 2017. Overall, 11 studies reported data on ovarian damage, 8 on uterine volume related to treatment, and 3 on vaginal effects. Most studies did not report accurate dosimetric data and dose-volume relationships, and the confounder role of chemotherapy was not considered. In general, published data confirmed that ovarian sensitivity to RT increases with age. Follicle sensitivity followed a linear model, with 2 Gy representing the lethal dose (50% of primordial follicles destroyed). The "effective sterilizing dose" (residual oocyte population $<1000$) decreased as age increased.

The Pediatric Normal Tissue Effects in the Clinic (PENTEC) task force produced this comprehensive review with the purpose of quantifying the effects of RT dose to the female reproductive organs after treatment for childhood cancer. They recommend to minimize RT dose and (if possble) to preserve one ovary, considering that the maximum tolerable RT dose decreases with patient's age and total dose of alkylating agents.

Several studies have defined acute ovarian failure (AOF) as the loss of oestrogen production within 5 years from RT, and premature ovarian insufficiency (POI) when the loss of oestrogen production and oocyte reserve become evident later during follow up, but before 40 years of age. The 'Dovary' model designed by the Childhood Cancer Survivorship study and the St. Jude Lifetime (SJLIFE) cohort to predict AOF showed that the risk of AOF increased with the mean RT dose to the least affected ovary: cyclophosphamide equivalent dose of alkylating agents (CED), and age at RT. The model designed by SJLIFE study to predict POI reported that the risk of POI increased with survivor age, Dovary, and dose of alkylating agents.

Uterine toxicity, in term of reduced volume, impaired arterial blood flow, fibrosis, endometrial dysfunction, early pregnancy loss, preterm birth and delivery of low-birthweight infants has been described in some studies. Available data were insufficient to design a model of RT-associated risk, but in general a small uterine volume was described when RT doses $>12$ Gy were used in younger patients.

Data on vaginal toxicity are scant: vaginal dryness, mucosal thinning, and fibrous stenosis up to complete closure requiring surgical procedures have been described. Similarly to adults, RT risk was confirmed to be related to dose ($>5$ Gy in adults) and field of application.

## 9.3. Therapeutic exposures and pubertal testicular dysfunction are associated with adulthood milestones and paternity after childhood cancer

Korhonen M, Tainio J, Koskela M, Madanat-Harjuoja LM, Jahnukainen K
kirsi.jahnukainen@helsinki.fi,
*Cancer.* 2023 Nov 15;129(22):3633-3644.
doi: 10.1002/cncr.34971. PMID: 37552054

Brief Summary: This cross-sectional study aimed to define if childhood cancer was related to delayed achievement of psychosocial milestones in later life.

Data of 252 male childhood cancer survivors (CCS) diagnosed with haematological or solid tumours between 1964 and 2000, with 6-42 years of survival, were collected and compared with 5 matched controls for each patient. CCS moved away from their parental home as frequently as population controls but were less likely to marry or live in a registered relationship, especially when they had been diagnosed with cancer at less than 4 years of age. CCS were less likely to sire a child and more likely to adopt. Lower probability of paternity was associated with hematopoietic stem cell transplantation, testicular radiation dose $>6$ Gy, laboratory signs of testicular dysfunction (FSH $>15$ IU/L, LH $>15$ IU/L, testosterone $<2$ ng/mL (5 nmol/L), need for induced puberty, testicular volume $<12$ mL at the end of puberty, and azoospermia in young adulthood.

This study emphasizes the need of monitoring pubertal development in male CCS because clinical and laboratory signs of testicular failure during adolescence potentially impact the achievement of psychosocial milestones and probability of paternity in adulthood.

## 9.4. Evaluating testicular tissue for future autotransplantation: focus on cancer cell contamination and presence of spermatogonia in tissue cryobanked for boys diagnosed with a hematological malignancy

Kourta D, Camboni A, Saussoy P, Kanbar M, Poels J, Wyns C
ann.boekstegers@med.uni-muenchen.de,
*Hum Reprod*. 2024 Mar 1;39(3):486-495.
doi: 10.1093/humrep/dead271. PMID: 38227814

Brief Summary: This retrospective cohort study included 54 pre- or peri-pubertal boys affected by hematological cancer who underwent a testicular biopsy for fertility preservation at the time of diagnosis, before any gonadotoxic treatment. The presence of cancer cells in immature testicular tissue of young boys was assessed by histology, immunohistochemistry (IHC) and PCR at the time of cryopreservation, before treatment. Contamination by cancerous cells was found in 10/28 boys using IHC, with a higher rate in patients with acute lymphoblastic leukemia, compared to those affected by lymphoma. PCR showed contamination in 3/15 patients who had specific bone marrow rearrangements at the time of diagnosis. Mean spermatogonial number was decreased in patients of all age groups when compared to healthy reference cohorts.

In past decades, the prepubertal testis was considered a preferential site for leukemia and lymphoma relapse. Testicular biopsy was used to assess the presence of residual cancer cells, as an indicator of treatment effectiveness and recurrence risk. Tissue fragments were analysed with histology and immunohistochemistry and cancer cell contamination ranged from 15% to 45%. The results of these analyses indicated the spreading potential of cancer cells and their ability to persist in the testis after treatment. However, it is not directly comparable with assessment of cancer cell contamination of cryopreserved testicular tissue taken for fertility preservation.

This is the first study to demonstrate a high risk of contamination by cancer cells in immature tissue collected before any therapy and analyzed with modern techniques. In clinical practice, it is essential to identify patients who might benefit from the cryopreservation without relapse risk. The risks of contamination have led many groups to collect immature testicular tissue after the first cycles of chemotherapy, as in prepubertal and adult females this modality is shown to reduce ovarian tissue contamination by cancer cells, without altering fertility potential.

Reference

1. Kourta D, Kanbar M, Amorim CA, Wyns C. Cancer cell contamination and decontamination methods for ovaries and testes: special focus on prepubertal gonads with a view to safe fertility restoration. *Hum Reprod* 2023; 38:780–798.

## 9.5. Fertility potential and gonadal function in survivors of reduced-intensity hematopoietic stem cell transplantation

Rotz SJ, Hamilton BK, Wei W, Ahmed I, Winston SA, Ballard S, Bernard RJ, Carpenter P, Farhadfar N, Ferraro C, Friend BD, Gloude NJ, Hayashi RJ, Hoyle K, Jenssen K, Koo J, Lee CJ, Mariano L, Nawabit R, Ngwube A, Lalefar N, Phelan R, Perkins L, Rao A, Rayes A, Sandheinrich T, Stafford L, Tomlinson K, Whiteside S, Wiedl C, Myers K
rotzs@ccf.org,
*Transplant Cell Ther.* 2024 May; 30 (5):534.e1-534.e13.
doi: 10.1016/j.jtct.2024.02.002. PMID: 38342136

Brief Summary: This multicenter, international, retrospective study evaluated fertility potential and gonadal function in 326 adolescent and young adult cancer survivors. Risk factors for impairment included

hematopoietic stem cell transplantation (HCT) conditioning regimen (myeloablative conditioning, MAC vs reduced-intensity conditioning, RIC).

The prevalence of gonadal hormone failure in females was 55.3% (defined as FSH $>30$ mIU/mL with an estradiol $<17$ pg/mL or current use of hormone replacement therapy). Only 1/45 female recipients of MAC and 4/26 recipients of RIC had preserved fertility potential (AMH $\geq 0.5$ ng/mL). Older age at HCT was associated with greater likelihood of gonadal failure, while conditioning intensity, total body irradiation (TBI), chronic graft-versus host disease (GVHD) requiring systemic therapy and cyclophosphamide equivalent dose (CED) were not associated with gonadal function. The incidence of gonadal failure in males was 44% (defined as FSH $>10.4$ mIU/mL or current use of hormone replacement therapy). Older age (median, 16.2 vs 14.4 years) and TBI dose were associated with gonadal failure, whereas conditioning intensity and CED were not significantly associated.

RIC regimens are increasingly used to decrease end-organ toxicity from HCT. However, the results here suggest that RIC does not substantially mitigate the risk for gonadal failure in either sex. More RIC recipients had detectable AMH levels and all 4 females with spontaneous pregnancy had a nonmalignant condition and received RIC, but these successes were infrequent.

Study limitations include the small number of patients with measured hormone levels and the short follow up (1 to 2 years post-HCT). It is possible that differences in AMH levels between RIC and MAC recipients might only emerge during a longer follow-up. The authors did not account for pubertal stage at the time of HCT, and therefore patients who remained prepubertal 1 to 2 years post-transplantation might have spuriously low FSH levels (despite substantial gonadal damage). Only 7 males had post-HCT semen analysis, which is the gold standard for assessing fertility in HCT male survivors. Further studies investigating the fertility outcomes of newer, specific conditioning regimens with reduced-toxicity (eg, treosulfan-based) are needed.

## 9.6. Reproductive outcomes and reproductive health care utilization among male survivors of childhood cancer: a DCCSS-LATER study

Claessens JJM, Penson A, Bronkhorst EM, Kremer LCM, van Broeder 7 E, van der Heiden-van der Loo M, Tissing WJE, van der Pal HJH, Blijlevens NMA, van den Heuvel-Eibrink MM, Versluys AB, Bresters D, Ronckers CM, Walraven I, Beerendonk CCM, Loonen JJ, Dutch later study group.
joyce.claessens@radboudumc.nl,
*Cancer.* 2024 Mar 15;130(6):995-1004.
doi: 10.1002/cncr.35119. PMID: 38055238

Brief Summary: The nationwide Dutch Childhood Cancer Survivor (CCS) LATER cohort study assessed by questionnaire the reproductive outcomes and reproductive health care in 1317 male CCS and 407 male siblings. This included 491 CCS and 185 siblings who expressed a previous or current desire for children. Fewer CCS than siblings reported having biological children (65% vs. 88%). The type of conception was similar between CCSs and siblings (spontaneous conception in 90% of both groups). More CCS than siblings had consulted a reproductive specialist for infertility (34% vs. 12%). But fewer CCSs underwent assisted reproductive techniques (ART) (41% vs. 77%), and even when received, ART had a lower success rate (to father a child) (49% vs. 94%). Surgical sperm retrieval procedures were performed in only 10 CCS, but none of their partners conceived.

Gonadal failure is the most common late endocrine effect of cancer treatment. Previous studies reported that only few CCS sired a pregnancy (15%–30%). The current study focused only on men who wanted children, which could explain the overall better success rate (65%) than previously. Male CCS were almost three times more likely to consult a reproductive specialist for difficulty conceiving compared to siblings, but the use of ART following consultation was less frequent in CCS than in their healthy siblings.

The study provided no insight into details concerning ART that could clarify the potential mechanisms behind the observed difference. Fertility assessment predicting a good fertility potential has been hypothesized, but an additional reason for the lower use of ART might have been the finding of azoospermia on semen analysis. Previous studies on testicular sperm extraction (TESE) reported sperm retrieval rates of 36% in cancer survivors

and live birth rates of 42% to 53% obtained with ICSI after successful TESE. Data are needed on the success rate of TESE in CCS with azoospermia and factors associated with successful sperm retrieval to inform the value of this technique.


Reference

1. Hsiao W, Stahl PJ, Osterberg EC, Nejat E, Palermo GD, Rosenwaks Z, Schlegel PN. Successful treatment of postchemotherapy azoospermia with microsurgical testicular sperm extraction: the Weill Cornell experience. *J Clin Oncol*. 2011 Apr 20;29(12):1607-11. doi: 10.1200/JCO.2010.33.7808. Epub 2011 Mar 14. PMID: 21402606.


## 9.7. Reproductive ability in survivors of childhood, adolescent, and young adult Hodgkin lymphoma: a review


Drechsel KCE, Pilon MCF, Stoutjesdijk F, Meivis S, Schoonmade LJ, Wallace WHB, van Dulmen-den Broeder E, Beishuizen A, Kaspers GJL, Broer SL, Veening MA

k.c.e.drechsel@amsterdamumc.nl,



*Hum Reprod Update*. 2023 Jul 5;29(4):486-517.

doi: 10.1093/humupd/dmad002. PMID: 36779325



Brief Summary: This narrative review summarized 75 published studies on reproductive function in survivors of childhood, adolescent, and young adult Hodgkin lymphoma (HL). 41 papers reported on 5057 female HL survivors. Most young female survivors had regular menstrual cycles. The incidence of premature ovarian failure (POI) varied between 6-34%. Biochemical evidence for impaired ovarian reserve or function was frequent (low AMH 55–59%. elevated FSH 17–100%). 51 studies assessed fertility in 1903 male HL survivors. Post-treatment azoospermia was common (33–100%). A few studies reported recovery of semen up to 12 years post-treatment. Elevated FSH and low inhibin B levels were also common (elevated FSH 0–100%; low inhibin B 19–50%). LH and testosterone levels were less affected (elevated LH 0–57%; low testosterone 0–43%).

In both sexes, impaired reproductive ability was associated with pelvic radiotherapy and a higher cumulative chemotherapy doses. However, an impaired ovarian reserve did not exclude the chance of a live birth, and males with aberrant gonadal markers could still conceive. The presence of markers of impaired fertility before treatment (low AMH in females and azoospermia in males) indicates that the disease itself may disrupt reproductive function.

These findings indicate the need for longitudinal follow up with repeated measurements and a combination of reproductive markers (semen analysis, FSH and inhibin B in males; and AMH, antral follicle count and FSH in females) in all young adults affected by HL. Treatment related parameters such as abdominal radiotherapy, CED score (cyclophosphamide equivalent dose), using the cutoff $>6000$ mg/m2 in girls and $>4000$ mg/m2 in boys, and age at diagnosis (pre-/post pubertal) should be always assessed. The review also highlights that the adverse effects of newer/novel drugs that are believed to be non-gonadotoxic (e.g., Brentuximab, Nivolumab, and Pembrolizumab) are still largely unknown.



References

1. Mulder RL, Font-Gonzalez A, Green DM, Loeffen EAH, Hudson MM, Loonen J et al. Fertility preservation for male patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE Consortium and the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol* 2021;22: e57–e67.
2. Mulder RL, Font-Gonzalez A, Hudson MM, van Santen HM, Loeffen EAH, Burns KC et al. Fertility preservation for female patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE Consortium and the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol* 2021;22: e45–e56.


## 9.8. Perceived and objective fertility risk among female survivors of adolescent and young adult cancer


Din HN, Singh-Carlson S, Corliss HL, Hartman SJ, Strong D, Madanat H, Su HI

hisu@health.ucsd.edu,



*JAMA Netw Open*. 2023 Oct 2;6(10):e2337245.

doi: 10.1001/jamanetworkopen.2023.37245. PMID: 37819662

Brief Summary: This retrospective cohort study assessed whether estimated treatment gonadotoxicity and posttreatment menstrual patterns are associated with higher infertility risk perception in a group of survivors of adolescent or young adult cancer (age 15-39 years).

Participants reported their menstrual pattern and infertility risk perception and were categorized as increased risk (feeling less fertile or unable to become pregnant) or no increased risk (feeling fertile) compared with control female individuals of similar age. Basal ovarian function and reserve were assessed by hormone levels measured on a self-collected dried blood spots.

Overall, 417/654 (63.8%) accurately assessed their risk; 96 (14.7%) had no objective or perceived increased risk, and 321 (49.1%) had objective and perceived increased risk. 83 participants (12.7%) overestimated their risk, and 154 (23.5%) underestimated their risk. Prior exposure to treatments with moderate or high gonadotoxicity was associated with higher odds of perceiving increased infertility risk. Amenorrhea and irregular cycles were associated with higher odds of perceiving increased infertility risk. Multiparity was associated with increased odds of underestimation, while older age, endocrine comorbidity and prior infertility were associated with lower odds of underestimation. Multiparity, breast cancer and skin cancer were associated with lower odds of overestimation.

Many cancer survivors experience fertility problems, but data on their perceptions and awareness of their fertility issues and their adjustment with objective infertility risk are scarce. Risk perceptions directly influence health behaviours, medical information seeking and treatment compliance. This study confirms that cancer survivors perceive increased infertility risk, many are able to appropriately estimate its degree. Counselling on infertility risk throughout survivorship is needed to reduce misalignment between perceptions and actual risk, decrease psychological distress, and correctly inform family planning decisions.

## 9.9. Risk of adverse birth outcomes after adolescent and young adult cancer

Anderson C, Baggett CD, Engel SM, Getahun D, Cannizzaro NT, Mitra S, Meernik C, Moy LM, Laurent CA, Zhou X, Xu L, Kwan ML, Wood WA, Luke B, Chao CR, Kushi LH, Nichols HB
chelsea_anderson@med.unc.edu,
*JNCI Cancer Spectr.* 2024 Jan 4;8(1): pkad106.
doi: 10.1093/jncics/pkad106. PMID: 38127994

Brief Summary: This retrospective cohort study analysed risk ratios for preterm birth (< 37 completed weeks), very preterm birth (< 34 completed weeks), low birth weight (< 2500 g), and small for gestational age (SGA, < 10th percentile of weight for gestational age) in a large group of women diagnosed with adolescent and young adult (AYA, age 15-39 years) breast cancer, thyroid cancer, gynaecologic cancers, lymphoma, or melanoma.

1648 post-cancer births were each matched to 5 births of women without cancer. Overall, the risks of preterm birth, very preterm birth, low birth weight, and SGA did not differ between births to AYA survivors and women without cancer. However, women with gynaecologic cancers had increased risk of low birth weight and a slightly increased risk of preterm birth. Chemotherapy exposure was not associated with risk of adverse birth outcomes. In stratified analyses, the risk of preterm birth to women who smoked during pregnancy was increased for AYA cancer survivors compared to women without a cancer history. AYA cancer survivors with a pre-pregnancy obesity also had an increased risk of preterm birth and low birth weight, compared to women with a similar pre-pregnacy BMI but without a history of cancer.

Cancers diagnosed among AYA occurs in a critical life period where decisions for childbearing and parenthood are made. With a 5-year survival rate > 85% for all cancers combined in this age group, many AYA women will live long enough to have biological children, and be impacted by the effects of their cancer treatment on their reproductive outcome. Previous studies, mostly including women diagnosed with cancer prior to the year 2000, reported a higher rate of preterm births and low birth weight among cancer survivors compared to the general population. This study does not confirm such risks of adverse birth outcomes, except for AYA survivors of gynaecologic cancers or those who carry additional risk factors, such as obesity or smoking.

Given the relatively small sample size, the authors could not conduct more specific analyses for cancer types (cervical, ovarian, uterine). However, the increased risk due to gynecological cancers is highly plausible, given the anatomical location of these malignant tumors and their treatments (pelvic surgery and/or radiation therapy).

References

1. van der Kooi ALF, Brewster DH, Wood R, et al. Perinatal risks in female cancer survivors: a population-based analysis. *PLoS One*. 2018;13(8): e0202805.
2. Huang W, Sundquist K, Sundquist J, Ji J. Risk of being born preterm in offspring of cancer survivors: a national cohort study. *Front Oncol*. 2020; 10:1352

## Thyroid Issues in Cancer Survivors

### 9.10. Primary hypothyroidism in childhood cancer survivors treated with radiation therapy: a PENTEC comprehensive review

Milano MT, Vargo JA, Yorke ED, Ronckers CM, Kremer LC, Chafe SMJ, van Santen HM, Marks LB, Bentzen SM, Constine LS, Vogelius IR
Michael_Milano@urmc.rochester.edu,
*Int J Radiat Oncol Biol Phys*. 2024 Jun 1;119(2):482-493.
doi: 10.1016/j.ijrobp.2021.02.001. PMID: 33810948

Brief Summary: This review analysed 15 original studies reporting the effects of radiation therapy (RT) on thyroid gland in childhood cancer survivors (CCS). A relationship between RT dose to the thyroid gland and risk of hypothyroidism could be extrapolated from 8/15 studies. Risk of compensated hypothyroidism increased with RT dose to the thyroid: it was 12%, 25% and 44% at 10, 20 and 30 Gy, respectively. Similarly, the risk of overt hypothyroidism was 4%, 7% and 13% at 10, 20 and 30 Gy, respectively. There was no clear lower threshold RT dose of safety. Age $>15$ years at RT and female sex were associated with increased risk of hypothyroidism.

The thyroid gland is often an incidental target of RT in CCS, resulting in various functional impairments, nodularity and/or malignancy. Patients at risk for RT-induced hypothyroidism should undergo regular monitoring of thyroid function, physical examination and ultrasound imaging to assess the emergence of nodules. Hypothyroidism occurs generally within the first 5 years after RT, but some studies report a precocious onset when high RT doses were used, while other studies reported a later onset 5 to 10 years after RT. This study confirmed a RT dose-response relationship with the risk of hypothyroidism, without any safe lower threshold dose. For this reason, thyroid RT exposure should be minimized when feasible, and regular monitoring should be performed in all CCS exposed to RT.

### 9.11. Thyroid ultrasound screening in childhood cancer survivors following radiotherapy

Baran JA, Halada S, Bauer AJ, Li Y, Isaza A, Patel T, Sisko L, Ginsberg JP, Kazahaya K, Adzick NS, Mostoufi-Moab S
moab@chop.edu,
*Horm Res Paediatr*. 2024;97(3):243-253.
doi: 10.1159/000531241. PMID: 37722360

Brief Summary: This retrospective study assessed the outcome of thyroid ultrasound (US) surveillance in childhood cancer survivors (CCS) exposed to radiotherapy (RT). 306 CCS were monitored with thyroid US. Patients received TBI (45%) and/or RT to craniospinal (44%), chest (11%), and neck regions (6%). Thyroid US surveillance was started at a median interval of 9.1 years after RT; 150 patients (49%) had thyroid nodule(s); 44 patients underwent surgery, and 28 had a final diagnosis of differentiated thyroid cancer (DTC).

There was no difference in the median radiation dose between CCS with or without thyroid nodules. Eight patients with intermediate- or high-risk disease received RT at age $\leq 10$ years but only 1 in those who recived

RT at age $>10$ years. RT at age $\leq 3$ years old conferred a 2.87-fold increased risk for nodule presentation compared to RT at $>10$ years old. Female sex and longer duration between RT and first US were additional independent risk factors for thyroid nodule(s).

Thyroid nodules and DTC are among the most common late effects of RT. Estimated lifetime risk for developing DTC among CCS who received radiotherapy (RT) varies greatly, with standardized incidence ratios between 5 and 69-fold higher, compared to controls. Screening recommendations for secondary DTC remain controversial: the current Children's Oncology Group Follow-Up Guidelines recommend annual neck palpation and conservative US use, only for palpable nodules. On the contrary, endocrine professional societies advocate for more precise monitoring by thyroid US, regardless of neck palpation findings.

This study suggests that thyroid US surveillance is particularly useful in CCS exposed to RT in early childhood. Prospective studies are needed to further define the latency between RT exposure and nodule development and to produce tailored thyroid surveillance guidelines for CCS, avoiding delayed diagnosis without an increase in aggressive and unnecessary interventions.

References

1. Clement SC, Kremer LCM, Verburg FA, Simmons JH, Goldfarb M, Peeters RP, et al. Balancing the benefits and harms of thyroid cancer surveillance in survivors of Childhood, adolescent and young adult cancer: recommendations from the international Late Effects of Childhood Cancer Guideline Harmonization Group in collaboration with the PanCareSurFup Consortium. *Cancer Treat Rev*. 2018; 63: 28–39.
2. Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedus L, et al. American association of clinical endocrinologists, American college of endocrinology, and associazione medici endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules - 2016 update. *Endocr Pract.* 2016;22(5): 622–39

## 9.12. Risk of second primary thyroid cancer in cancer survivors

Yuan Y, Sun P, Xiao H, Li L, Li J, Ai X
lijunyan4990@126.com; axtmmu@163.com,
*Sci Rep*. 2024 May 30; 14(1):12478.
doi: 10.1038/s41598-024-63155-z. PMID: 38816510

Brief Summary: This retrospective study evaluated the risk and clinicopathological features of second primary thyroid cancer (SPTC) in cancer survivors. The Surveillance, Epidemiology, and End Results (SEER) Program collected cancer incidence and mortality data from 8 population-based registries. They identified 7066 patients with SPTC and 83,113 patients with primary thyroid cancer (TC). The standardized incidence ratio (SIR) of SPTC in cancer survivors was higher than in the general population (1.51 vs 0.94/10,000).

The risk of SPTC was associated with some first tumors (acute lymphocytic leukemia, Hodgkin's lymphoma, salivary gland cancer, kidney cancer) and radiotherapy/chemotherapy before age 35 years. Patients with SPTC were younger (49.6 years vs 64), with a higher proportion of males (34% vs 25%). The mean latency between first tumor and SPTC was 7.6 years, but differed by type of first tumor. Primary lung and bronchus tumors were associated with early SPTC, while median latency between Hodgkin's lymphoma and SPTCs was 16 years. The most common pathological type was papillary carcinoma in both groups, with a higher percentage of histological grades 3/4 in cancer survivors (23% vs. 15%). No difference in survival rate was found between SPTC and primary TC, after adjusting for other factors.

The present study compared demographics and clinicopathological characteristics of primary TC and SPTC in a very large dataset. There was an increasing trend of thyroid cancer diagnosis with time, probably due to improvements in detection methods as well as more regular surveillance. The study showed for the first time that first tumor survivors who received chemotherapy (not only radiotherapy) before age 35 years had a significantly increased incidence of thyroid cancer. Limitations of the study are its retrospective design; it was impossible to verify missing information from the SEER database, and data on primary tumor treatment were limited to 'yes/no' radiotherapy and chemotherapy. However, these data, including the latency between the first tumor diagnosis and SPTC, should contribute to improved surveillance strategies.

## 9.13. GH and childhood-onset craniopharyngioma: when to initiate GH replacement therapy?

Nguyen Quoc A, Beccaria K, González Briceño L, Pinto G, Samara-Boustani D, Stoupa A, Beltrand J, Besançon A, Thalassinos C, Puget S, Blauwblomme T, Alapetite C, Bolle S, Doz F, Grill J, Dufour C, Bourdeaut F, Abbou S, Guerrini-Rousseau L, Leruste A, Brabant S, Cavadias I, Viaud M, Boddaert N, Polak M, Kariyawasam D
adrien.nguyenquoc@aphp.fr,
*J Clin Endocrinol Metab*. 2023 Jul 14;108(8):1929-1936.
doi: 10.1210/clinem/dgad079. PMID: 36794424

Brief Summary: This retrospective, observational, single centre study compared the risk of tumor progression or recurrence in 71 patients with childhood-onset craniopharyngioma (CP) who started GH replacement therapy (GHRT) with different latency from the end of tumor treatment.

GHRT latency was defined as the time from end of CP treatment (last debulking procedure or radiotherapy, or from CP diagnosis for the 5 patients without any debulking procedure or radiotherapy) to start of GHRT. 27 patients had a GHRT latency 12+ months (median 17 months); while 44 patients had a GHRT latency <12 months (median 7 months, and between 6-12 months in 29 patients). Event-free survival rates at 2- and 5-years were similar between groups (82% and 69% in the >12-month group; 72% and 70% in the <12-month group; 72% and 72% in the 6-12-month group). The risk of CP new events was not associated with GHRT latency. The median final height, achieved by 41 patients, was higher in the >12 months group (males 185 cm, females 165 cm) than in the <12 months group (males 177 cm, females 163.5 cm).

This study suggests that the risk of relapse or progression of craniopharyngiomas is not influenced by the latency before starting growth hormone replacement therapy (GHRT). The authors conclude that GHRT can be initiated 6 months after the last treatment. The higher final height in the >12 months group may be related to younger age at diagnosis, allowing more prolonged GHRT, or confounded by the indication to start GHRT sooner in shorter patients.

Strengths of this study are the homogeneous cohort and the long duration of follow-up (8 years). The main weakness is the low number of events, reflecting the rarity of CP. In light of recent reassuring evidence on the safety of GHRT in craniopharyngiomas, prospective studies on larger cohorts are needed not only to support these findings but also to assess the potential negative effects of delayed GHRT on body composition and physical and psychological well-being.

Reference

1. Boguszewski MCS, Boguszewski CL, Chemaitilly W, et al. Safety of growth hormone replacement in survivors of cancer and intracranial and pituitary tumours: a consensus statement. *Eur J Endocrinol.* 2022; 186 (6): P35-P52. doi: 10.1530/EJE-21-1186

## 9.14. Exploring height outcomes with adjuvant aromatase inhibition in growth hormone-deficient male survivors of childhood cancer

Pollock NI, Song M, Wolf AJ, Li Y, Hawkes CP, Motamedi N, Denburg MR, Mostoufi-Moab S
pollockn@chop.edu,
*Pediatr Blood Cancer* 2024 Aug; 71(8):e31117.
doi: 10.1002/pbc.31117. PMID: 38804882

Brief Summary: This single-center, retrospective cohort study compared the final adult height (FAH) of 92 male childhood cancer survivors (CCS) with growth hormone deficiency GHD) treated with growth hormone alone (monotherapy) or in combination with an aromatase inhibitor. The addition of AI to GH therapy did not improve FAH.

This study from the Children's Hospital of Philadelphia is the most extensive study on the role of AI associated with GH in improving final height of CCS with growth hormone deficiency. A few previous studies had reported a slight benefit of AI in augmenting predicted adult height in boys with short stature.

70 CCS received GH monotherapy and 22 received GH plus aromatase inhibitors (GH + AI). There were no baseline differences in age at GH start, height Z-score, mid-parental Z-score, pubertal maturation (73% were prepubertal at initiation in GH group vs 50% in GH + AI group). But median age at cancer diagnosis was lower in the GH + AI group (3.2 vs 5.5 years). The most common diagnosis was neuroblastoma in the GH + AI group and CNS tumor in the GH group. More patients in the AI + GH group received stem cell transplantation, abdominal radiation, total body irradiation, and cis-retinoic acid. Mean FAH and FAH z-score were similar in the two groups (FAH 161 cm in GH + AI group vs 167 cm in GH group; FAH z-score -2.1 in AI/GH group vs -1.3 in GH group).

Increase in height z-score was higher in the GH group than the GH + AI group (mean 0.63 vs -0.30). However, the difference between FAH and MPH was higher in AI + GH group (median -19.1 vs -7.3 cm). Lower FAH z-score was associated with spinal radiation, lower height z-score at therapy start and greater difference between bone age and chronological age. No side effects were reported, including differences in glycated hemoglobin pre- and post-AI treatment.

Study limitations include the retrospective design, the small sample size, and differences in cancer treatments between the GH and GH + AI groups. Furthermore, selection bias is very likely, as AI was more likely indicated in patients with severe short stature and worse predicted final height. More patients in the GH + AI group received spinal radiation or cis-retinoic acid, which are known to negatively affect linear growth. Larger studies are needed to evaluate predicted height variation before and after AI and final adult height. At the same time, the AI side effect profile must be carefully considered in this population with a well-known increased risk of impaired glucose metabolism, cardiovascular events, hepatotoxicity and osteoporosis.

## 9.15. Association between conditioning intensity and height growth after allogeneic hematopoietic stem cell transplantation in children

Uemura S, Hasegawa D, Kishimoto K, Fujikawa T, Nakamura S, Kozaki A, Saito A, Ishida T, Mori T, Ozaki K, Kosaka Y
sgr3u31@yahoo.co.jp,
*Cancer Med*. 2023 Aug; 12(16):17018-17027.
doi: 10.1002/cam4.6336. PMID: 37434385

Brief Summary: This retrospective, single-center study from Kobe Children's Hospital, Japan, analyzed height SDS (main outcome) and risk of short stature (height < -2 SDS, secondary outcome) in 89 children with malignant diseases who underwent initial allogenic hematopoietic stem cell transplantation (HSCT) with different conditioning intensities. More intensive conditioning conferred a substantially higher risk of short stature at 3 years after HSCT.

58 patients received myeloablative conditioning (MAC, comprising TBI at > 8 Gy and busulfan > 8 mg/kg). 31 patients received reduced intensity conditioning (RIC). Median age at HSCT was lower in the RIC group (2.0 years) than MAC (9.3 years). Acute lymphoblastic leukemia (ALL) was the most common primary disease in the MAC group (59%), but not in the RIC group (16%). MAC was associated with a marked increase in the risk of short stature at 3 years after HSCT (adjusted OR: 5.6). Patients with and without ALL were compared, because corticosteroids suppress GH secretion and they still represent key drugs for ALL treatment. No difference was found between the ALL and non-ALL groups in height SDS at HSCT, and 1, 2, 3, 4, and 5?years after HSCT. No correlation was found between age at HSCT or total dose of TBI and height SDS at different time points.

RIC regimens, a well-recognized approach for allogenic HSCT for malignant and non-malignant diseases, aim to decrease short- and long-term complications induced by MAC. In this interesting study, RIC regimen reduced the risk of short stature after HSCT. Height SDS at 2 and 3 years after HSCT was lower in the MAC group, but significant differences in the height SDS at 4 and 5 years after HSCT between the two groups were not observed, probably due to the small number of patients at those times.

There are some limitations: the design is retrospective and selection bias is plausible because physicians may have avoided TBI in younger children. Moreover, the primary diseases were different in the MAC and RIC

groups, and patients may have received treatments with a diffferent impact on height before HSCT. Finally, the sample size and the proportion of patients reaching final height during follow up were small, with no data available on bone age.

## Bone Health and Chronic Diseases

### 9.16. Vertebral body reshaping after fractures: an important index of recovery in glucocorticoid-treated children

Ma J, Siminoski K, Jaremko JL, Koujok K, Matzinger MA, Shenouda N, Wilson N, Cheng M, Alos N, Atkinson S, Cummings EA, Ho J, Rodd C, Sbrocchi AM, Stein R, Barr R, Cairney E, Dix DB, Fernandez CV, Grant R, Halton J, Israels S, Laverdière C, Lewis VA, Cabral DA, Huber A, Houghton K, Jurencak R, Lang B, Larché M, LeBlanc CMA, Miettunen P, Roth J, Scuccimarri R, Bell L, Blydt-Hansen T, Filler G, Feber J, Phan V, Smit K, Rauch F, Ward LM
lward@cheo.on.ca,
*J Clin Endocrinol Metab*. 2024 Feb 20; 109(3):e1225-e1237.
doi: 10.1210/clinem/dgad611. PMID: 37843393

Brief Summary: This prospective natural history study examined the timing and positive indicators for reshaping of vertebral fractures (VF) in glucocorticoid (GC)-treated patients with chronic diseases. Complete VF reshaping occurred in 82.3% of patients, with similar frequencies in all diagnosis groups (leukemia, rheumatic disorders and nephrotic syndrome) in a median time of 1.3 years. Likelihood of VF reshaping was positively related to higher lumbar spine bone mineral density (LS BMD) z-score, lower number of VF, lower cumulative GC exposure, lower spinal deformity index (SDI) and lower VF grade.

GC treatment is known to cause VF, that can persist over time and cause important sequelae in adult age, such as short stature, back pain and deformities. With its large sample-size and the annual radiographic assessment, this study provides essential information on the natural history of VF reshaping, identifying reshaping time and influencing factors. Reshaping is possible in children, even without treatment with bisphosphonate, as the paediatric skeleton is a highly dynamic structure.

### 9.17. Fracture risk among children and adolescents with celiac disease: a nationwide cohort study

Zacay G, Weintraub I, Regev R, Modan-Moses D, Levy-Shraga Y
yael.levy.shraga@gmail.com,
*Pediatr Res*. 2024 Jan; 95(1):386-392.
doi: 10.1038/s41390-023-02826-5. PMID: 37749190

Brief Summary: This retrospective study evaluated fracture risk among 2372 children and adolescents (59% females, aged 1-16) with biopsy-proven celiac disease (CD) compared to 11,860 children without CD matched by age, sex, socioeconomic status, and population sector (general Jewish population, ultra-orthodox Jews and Arabs). The overall fracture incidence rate was higher in the CD group (256 vs 165 per 10,000 patient-years).

Median age at the end of the follow up was 12.8 years; median follow-up was 5.5 years. Patients with chronic diseases that could impact bone health were excluded. The hazard ratio (HR) for fracture was 1.57 for the CD group compared to the matched group (1.47 for boys and 1.67 for girls). The CD group also showed higher HR for multiple fractures (1.67), fractures in the pre-diagnosis period (1.64), and fractures after CD diagnosis (1.52). For both groups, the most common site of fracture was the radius/ulna. There were no differences in the mean Z-scores for height, weight and BMI between children with or without fractures.

According to this interesting and well-designed Israeli study, children with CD showed an increased fracture risk both preceding and following CD diagnosis and treatment, corroborating previous data from a large population-based cohort study reporting that the risk of fracture remained elevated up to 20 years after CD diagnosis. The

persistence of increased fracture risk years after CD diagnosis could be explained by an incomplete intestinal healing due to incomplete adherence to gluten free diet (GFD) or by the lower quality of micronutrients in the GFD.

Strengths of this study are the large, homogeneous and well-matched population and the long follow-up. Limitations include the retrospective design, which entailed the extraction of information from an electronic database. Moreover, blood tests and anthropometric measurements at diagnosis were performed in a non-experimental setting according to the clinician's discretion; therefore, some of the data during the follow-up were missing. Lastly, there were no measurements of bone mineral density and other bone parameters at the time of the fracture. Prospective studies are needed to evaluate changes in bone quantity and quality after initiation of GFD in children with CD, in order to identify those at risk for persistent metabolic bone disease and prevent fractures.

## Reference

1. Ludvigsson, J. F., Michaelsson, K., Ekbom, A. & Montgomery, S. M. Coeliac disease and the risk of fractures – a general population-based cohort study. *Aliment Pharm. Ther.* 25, 273–285 (2007).

# 10. Type 1 Diabetes

M. Loredana Marcovecchio[1], Marie-Anne Burckhardt[2]

[1]Department of Paediatrics, University of Cambridge, Cambridge, UK; [2]Pediatric Endocrinology and Diabetology, University Children's Hospital Basel UKBB, and Department of Clinical Research, University of Basel, Basel, Switzerland

## Clinical Trials - New Treatments

### 10.1. Baricitinib and beta-cell function in patients with new-onset type 1 diabetes

Waibel M, Wentworth JM, So M, Couper JJ, Cameron FJ, MacIsaac RJ, et al.
*N Engl J Med*. 2023;389(23):2140-50.
PMID: 38055252

Brief Summary: This phase 2, double-blind, placebo-controlled trial, randomized 91 people (10-30 years-old) diagnosed with type 1 diabetes (T1D) within the previous 100 days to receive either oral baricitinib (n = 60) or placebo (n = 31) for 48 weeks. Baricitinib was safe and preserved the capacity of β-cells to secrete insulin.

Baricitinib is a Janus kinase (JAK) inhibitor blocking cytokine signalling and is already an effective disease-modifying treatment for other autoimmune diseases, such as alopecia areata and rheumatoid arthritis (1,2). It is a plausible treatment for T1D because a key pathophysiological feature of T1D is the JAK-mediated destruction of β-cells by CD8 + T cells. Preclinical data suggested JAK inhibitors might influence β-cell function by blocking this pathway (3).

*BANDIT (Baricitinib in New-onset Type 1 Diabetes)* was a phase 2 trial conducted in 4 Australian sites. It successfully achieved its primary endpoint, higher mixed-meal-stimulated C-peptide levels in the baricitinib vs. placebo group (0.65 vs. 0.43 nmol/L/min). Baricitinib slowed the decline in C-peptide known to occur after T1D diagnosis; in the placebo arm the decline was on average 30% vs. only 4% with baricitinib. Insulin requirements were lower in the group on baricitinib as well as glucose variability on continuous glucose monitoring. Of note, HbA1c was similar between groups, which could reflect a similar standard diabetes management through insulin therapy and glucose monitoring in both groups.

The study is limited by its relatively small sample size and Caucasian-predominant demographics. Nevertheless, it is the first trial demonstrating oral baricitinib as a safe and effective potential disease-modifying drug for T1D. Several immunotherapy trials have been conducted in people with T1D within the first 100 days from diagnosis, which is an important window to protect residual endogenous β-cell function. However, most other immunotherapy agents require intravenous or subcutaneous injections. Baracitinib has the advantage of its oral route of administration.

Next step, will be to confirm the trial findings in a larger, more diverse population with T1D, explore whether benefits persist over time, and assess whether some subgroups of T1D might benefit more from this treatment.

References

1. Nash P, Kerschbaumer A, Dörner T, Dougados M, Fleischmann RM, Geissler K, et al. Points to consider for the treatment of immune-mediated inflammatory diseases with Janus kinase inhibitors: a consensus statement. *Ann Rheum Dis.* 2021;80(1):71-87.
2. King B, Ohyama M, Kwon O, Zlotogorski A, Ko J, Mesinkovska NA, et al. Two Phase 3 Trials of Baricitinib for Alopecia Areata. *N Engl J Med.* 2022;386(18):1687-99.
3. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discov.* 2017;17(1):78.

## 10.2. Teplizumab and beta-cell function in newly diagnosed type 1 diabetes

Ramos EL, Dayan CM, Chatenoud L, Sumnik Z, Simmons KM, Szypowska A, et al.
*N Engl J Med*. 2023;389(23):2151-61.
PMID: 37861217

Brief Summary: This double-blind, multicenter trial randomized 328 children and adolescents (8-17 years-old) diagnosed with type 1 diabetes (T1D) within the past 6 weeks to receive either teplizumab or placebo for two 12-day courses. Teplizumab increased stimulated C-peptide levels after 1.5 years, indicating better preservation of β-cell function.

Teplizumab is a monoclonal antibody that binds to CD3 on the surface of T-cells, thereby reducing the immune response that leads to the destruction of pancreatic β cells. In November 2022, the U.S. Food and Drug Administration approved Teplizumab to delay the onset of stage 3 T1D (clinically manifested T1D) in asymptomatic individuals ($\geq$8 years-old) with stage 2 T1D ($\geq$2 islet auto antibodies and dysglycemia) (1). Approval was granted based on a phase 2 trial where Teplizumab, administered in one 14-day course, delayed the progression from stage 2 to stage 3 T1D by 32.5 months and improved β-cell function in high-risk relatives of individuals with T1D (2).

The current findings, along with those from other similar trials, support teplizumab as a promising and safe disease-modifying therapy for newly diagnosed stage 3 T1D (3). Common adverse events included lymphopenia, rash, and headache, which occurred primarily during or after the first few weeks of teplizumab administration and were self-limited. Of note, there was no difference in other endpoints, such as HbA1c, insulin requirements, time in target glucose range and clinically relevant hypoglycemic events.

This study highlights the importance of early interventions in T1D. Administering teplizumab soon after diagnosis maximizes its potential benefits, aligning with the concept that T1D is a progressive condition that can be altered by early treatment. Early use of immunomodulatory therapies may become a standard component of T1D management.

Limitations are the lack of racial diversity in the study population and the relatively low rate of ketoacidosis than is typical in newly diagnosed T1D (4). Future research should optimise treatment protocols, explore combination therapies, and investigate the long-term impact of β-cell preservation on diabetes complications. Translation of these findings into clinical practice will require careful consideration of safety, patient selection, and long-term efficacy.

References


1. Keam SJ. Teplizumab: First Approval. *Drugs* 2023;83:439-445
2. Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *N Engl J Med* 2019; 381: 603-13.
3. Kamrul-Hasan ABM, Mondal S, et al. Role of Teplizumab, a Humanized Anti-CD3 Monoclonal Antibody, in Managing Newly Diagnosed Type 1 Diabetes: An Updated Systematic Review and Meta-Analysis. *Endocr Pract.* 2024;30:431-40.
4. Piccini B, Schwandt A, Jefferies C, Kordonouri O, Limbert C et al. Association of diabetic ketoacidosis and HbA1c at onset with year-three HbA1c in children and adolescents with type 1 diabetes: Data from the International SWEET Registry. *Pediatr Diabetes.* 2020; 21(2):339-48.


## 10.3. Pleconaril and ribavirin in new-onset type 1 diabetes: a phase 2 randomized trial

Krogvold L, Mynarek IM, Ponzi E, Mork FB, Hessel TW, Roald T, et al.
*Nat Med*. 2023;29(11):2902-8.
PMID: 37789144

Brief Summary: The Diabetes Virus Detection (DiViD) Intervention phase 2, placebo-controlled, double-blind trial randomized 96 children and adolescents (age 6-15 years) with new-onset type 1 diabetes (T1D) to receive either the antivirals, Pleconaril and Ribavirin, (n = 47) or placebo (n = 49) for 6 months. The antiviral treatment improved β-cell function at 12 months, and it was well tolerated and safe.

Viral infections, particularly enteroviruses, have been implicated in the pathogenesis of T1D, by triggering the autoimmune response or damaging pancreatic β-cells directly [1]. Previous evidence of low-grade enterovirus

infection in pancreatic islets of people with newly diagnosed T1D suggested that persistent chronic infection could lead to progressive β-cell damage (1). Hence the hypothesis that eradication of such low-grade infection might improve insulin secretion after the onset of T1D.

The DiViD trial was conducted at 2 sites, Oslo University Hospital (Norway) and Steno Diabetes Center Copenhagen (Denmark). Combination treatment with Pleconaril and Ribavirin, which both have antiviral activity against enteroviruses, was chosen to broaden the antiviral effect and reduce the risk of emergent drug-resistant variants.

The antivirals showed promising efficacy along with a good safety and tolerability profile. The primary endpoint was endogenous insulin production after 12 months, assessed by 2-h serum C-peptide area under the curve (AUC) during a mixed meal tolerance test, as used in previous immunotherapy trials (2).

C-peptide AUC at 12 months was higher in the treatment than in the placebo group. In addition, a higher proportion of participants in the antiviral group had a peak stimulated C-peptide $>0.2$ pmol/ml, a clinically relevant threshold. Some differences in HbA1c were seen at 3 and 6 months but not 12 months; however, those initial differences could be due to hemolysis related to antiviral treatment, leading to falsely lower HbA1c levels.

These findings provide solid evidence for the use of antiviral agents in people recently diagnosed with T1D. Limitations of the study include its relatively small sample size, which was sufficient only for the primary endpoint, the small age range and restricted location at only 2 centres in Northern Europe. Further studies are needed to confirm these results in larger and more diverse populations, to optimize the antiviral combination and also consider antiviral vaccinations as a preventive strategy for T1D.

References

1. Dahl-Jørgensen K. Virus as the cause of type 1 diabetes. *Trends Mol Med.* 2024; 13:S1471-4914(24)00183-7.
2. Latres E, Greenbaum CJ, Oyaski ML, Dayan CM, Colhoun HM, Lachin JM, et al. Evidence for C-Peptide as a Validated Surrogate to Predict Clinical Benefits in Trials of Disease-Modifying Therapies for Type 1 Diabetes. *Diabetes.* 2024;73(6):823-833.

## Important for Clinical Practice

## 10.4. Demographic, clinical, management, and outcome characteristics of 8,004 young children with type 1 diabetes

Sandy JL, Tittel SR, Rompicherla S, Karges B, James S, Rioles N, et al.
*Diabetes Care*. 2024;47(4):660-7.
PMID: 38305782

Brief Summary: This observational study highlights the challenges of managing T1D in 8,004 young children with type 1 diabetes (T1D) (age $<$ 6 years) from 3 international registries: Diabetes Prospective Follow-Up Registry (DPV), T1D Exchange Quality Improvement Network (T1DX-QI), and the Australasian Diabetes Data Network (ADDN), using data collected between 2019 and 2021. More than half of included children did not achieve the recommended HbA1c target $<$ 7.0% (53 mmol/mol). While continuous glucose monitoring (CGM) was used by most participants, insulin pump use varied between the registries and hybrid closed loop (HCL) use was rare.

These data are alarming given the known association between suboptimal glycemic levels and the risk of acute and chronic complications (1). Furthermore, the study underscores disparities in the use of diabetes technologies, such as CGM and insulin pumps and particularly HCL systems, with lower use among children from low socioeconomic backgrounds. Despite strong evidence supporting the value of HCL systems in this young population (2,3), their use was uncommon across all three registries. This is most likely due to pending regulatory approvals in very young children during the study period (2019-2021). Use of HCL systems in this age group is progressively increasing and should lead to more young children achieving optimal glycemic targets. However, advocacy is essential to improve access to diabetes technologies for all young children with T1D in a timely and equitable manner.

In line with the increasing number of overweight/obese children in the general population, the study reported that a high percentage of T1D children had a BMI in the overweight range (36-50%), which may explain some of the variation in outcomes between the registries.

Although this study comprises a large sample size, all 3 registries cover primarily high-income countries and do not reflect the situation in middle- and low-income countries.

In conclusion, these findings underscores the complexity of managing T1D in young children and the need for personalized care and equitable access to advanced technologies.

References

1. de Bock M, Codner E, Craig ME, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Glycemic targets and glucose monitoring for children, adolescents, and young people with diabetes. *Pediatr Diabetes*. 2022;23(8):1270-1276.
2. Ware J, Allen JM, Boughton CK, et al.; KidsAP Consortium. Randomized trial of closed-loop control in very young children with type 1 diabetes. *N Engl J Med* 2022;386:209–219.
3. Wadwa RP, Reed ZW, Buckingham BA, et al.; PEDAP Trial Study Group. Trial of hybrid closed-loop control in young children with type 1 diabetes. *N Engl J Med* 2023;388:991–1001.

## 10.5. Equitable implementation of a precision digital health program for glucose management in individuals with newly diagnosed type 1 diabetes

Prahalad P, Scheinker D, Desai M, Ding VY, Bishop FK, Lee MY, et al.
*Nat Med*. 2024;30(7):2067-75.
PMID: 38702523

Brief Summary: This prospective, pragmatic, open-label study assessed the impact of a systematic and equitable digital-health-team-based care program designed to achieve tight glycemic targets (HbA1c $<7\%$) through early technology use and remote patient monitoring, in young people with newly diagnosed T1D. The program was successful: 68% of participants achieved target HbA1c, and an average 65% time in glucose range at one-year post-diagnosis.

Despite advances in diabetes management, still few young people with T1D meet recommended glucose targets (1). This is worrying given the known association between suboptimal glycemic target and risk of complications. Diabetes technologies, such as continuous glucose monitoring (CGM) and hybrid closed loop, enable better glycemic outcomes and quality of life (2). However, there are wide inequalities in access to these technologies, with low use among minority racial and ethnic groups, low socioeconomic status, and at low levels of public health insurance (3).

The US-based Teamwork, Targets, Technology and Tight Control study 1 (4T Study 1) used a population-based approach to promote wider and early access to technology and achieve glycemic targets (HbA1c $<7\%$) in young people with new onset T1D. A key aim of the program was to ensure early CGM initiation (within the first month of diagnosis) and remote patient monitoring. The intervention successfully achieved better HbA1c compared to a historical cohort study and a previous pilot study (4T study, 6.6%; pilot study, 7.2%; historical cohort, 7.7%). Nearly 70% of participants met the glycemic target HbA1c $<7\%$ at one-year after diagnosis.

This study highlights the positive impact of early implementation of technology and remote patient monitoring to create more equity in care delivery and allow timely resolution of difficulties due to technology use or suboptimal diabetes targets. It shows that financial support is needed to equip individuals with the required diabetes management tools. To allow equitable access, CGM were provided to all young people regardless of health insurance coverage. iPod Touch devices were offered to those without a compatible smartphone. The study also highlighted the key role of a multidisciplinary diverse team, able to engage with individuals from different ethnic and socio-economic backgrounds.

Overall, the study showed that population-based tools to prioritize care are essential to reduce inequities in diabetes care. Although tested in a selected setting, the 4T Study 1 model could be implemented and adapted in other clinical settings.

References

1. Gerhardsson P, Schwandt A, Witsch M, Kordonouri O, Svensson J, Forsander G, et al. The SWEET Project 10-Year Benchmarking in 19 Countries Worldwide Is Associated with Improved HbA1c and Increased Use of Diabetes Technology in Youth with Type 1 Diabetes. *Diabetes Technol Ther.* 2021;23(7):491-9.

2. Boughton CK, Hovorka R. The role of automated insulin delivery technology in diabetes. *Diabetologia.* 2024;67(10):2034-44.
3. Burckhardt MA, Addala A, de Bock M. Editorial: Equity in type 1 diabetes technology and beyond: where are we in 2022? *Front Endocrinol (Lausanne).* 2024;15:1400240.

## Clinical Guidance

## 10.6. Consensus guidance for monitoring individuals with islet autoantibody-positive pre-stage 3 type 1 diabetes

Phillip M, Achenbach P, Addala A, Albanese-O'Neill A, Battelino T, Bell KJ, et al.
*Diabetes Care*. 2024 Aug 1;47(8):1276-1298.
PMID: 38912694

Brief Summary: This is the first International Consensus Guidance on the monitoring of children, adolescents and adults with early-stage (pre-symptomatic) Type 1 Diabetes (T1D), defined as those individuals positive for islet autoantibodies and with either normoglycemia or dysglycemia.

With the progressive increase in screening programs for T1D around the world, there is a growing number of individuals identified with early-stages pre-symptomatic T1D (1). The detection of islet autoantibodies is currently the earliest indicator of future clinical T1D risk (2). Many individuals with positive T1D autoantibodies are offered monitoring through research studies and might be enrolled into intervention trials. However, not all individuals wish to or can participate in research. Still, they should be offered some form of follow-up to diagnose early on stage 3 T1D (symptomatic T1D) before onset of diabetic ketoacidosis (DKA) and to enable better long-term outcomes.

This landmark consensus guidance was developed by Breakthrough T1D (formerly known as JDRF) in partnership with several diabetes and endocrine societies and experts from several countries. It is designed for non-specialists, primary care providers, and pediatricians, as these are most likely to care for individuals who test positive for T1D autoantibodies. The guidance stresses that a partnership should be established between endocrinologists and primary care providers to care for these individuals. Key points are: 1) a positive screen with one or more T1D autoantibody should be confirmed by testing a second sample, to exclude false positive results; 2) those with confirmed early-stage T1D should have periodic medical monitoring, including 3-6 monthly glucose testing, regular education about symptoms of diabetes and DKA, and psychosocial support; 3) monitoring should be personalised based on age, number of autoantibodies and T1D stage (stage 1: normoglycemia; stage 2: dysglycemia); 4) people with pre-symptomatic T1D should be offered trial participation or approved immunotherapies, where available. Of note, the guidance outlines the importance of psychosocial and educational support for individuals and families and how this should be provided.

This guidance represents a key milestone in the field of T1D. It will help clinicians support the increasing numbers of children and young people being identified in early stages of T1D through general population screening or clinical practice.

References

1. Simmons KM, Sims EK. Screening and Prevention of Type 1 Diabetes: Where Are We? J Clin Endocrinol Metab. 2023;108(12):3067-79.
2. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *Jama.* 2013;309(23):2473-9.

## New Paradigms

## 10.7. Heterogeneity and endotypes in type 1 diabetes mellitus

Redondo MJ, Morgan NG
*Nat Rev Endocrinol*. 2023;19(9):542-54.
PMID: 37337007

Brief Summary: This review describes the heterogeneity in type 1 diabetes (T1D), the emerging concept of endotypes, and their impact on T1D prediction, prevention and treatment.

Growing evidence supports the existence of heterogeneity in T1D genetic background, pathogenesis, clinical course, susceptibility to complications and response to emerging immunotherapy (1,2). This has led to the concept that T1D is not a single disease; instead there are distinct subtypes known as 'endotypes' (1-3).

Previous studies have led to the characterization of two main endotypes: T1DE1 and T1DE2, which are associated with age at clinical diagnosis. T1DE1 includes cases of T1D diagnosed in early childhood and is characterized by a hyperimmune pattern of insulitis with high numbers of $CD8^+$ T and $CD20^+$ B cells, limited residual insulin-containing islets, abnormal proinsulin processing and an elevated circulating proinsulin-to-C-peptide ratio. In contrast, T1DE2 includes cases of T1D diagnosed in adolescence or adulthood and is characterized by more residual insulin-containing islets and without insulitis, fewer infiltrating $CD8^+$ T and $CD20^+$ B cells, normal proinsulin processing and lower proinsulin-to-C-peptide ratio than T1DE1.

There is emerging evidence that these 2 endotypes might respond differently to some immunotherapies. In particular, the T1DE1 might respond better than T1DE1 to agents directed to specific immune cell subsets, such as rituximab or teplizumab, while GAD–alum therapy might be effective for treating T1DE2.

Recognising T1D heterogeneity and further characterisation of endotypes could provide critical information for a more personalized approach and the design of more targeted immunotherapies to arrest or slow T1D progression.

References


1. den Hollander, N.H.M. and B.O. Roep, From Disease and Patient Heterogeneity to Precision Medicine in Type 1 Diabetes. *Front Med (Lausanne)*. 2022;9:932086.
2. Battaglia, M., et al., Introducing the Endotype Concept to Address the Challenge of Disease Heterogeneity in Type 1 Diabetes. *Diabetes Care*. 2020;43(1):5-12.
3. Parviainen, A., et al., Heterogeneity of Type 1 Diabetes at Diagnosis Supports Existence of Age-Related Endotypes. *Diabetes Care*. 2022;45(4):871-879.


## New Genetic Insights

### 10.8. Familial aggregation and heritability of childhood-onset and adult-onset type 1 diabetes: a Swedish register-based cohort study


Wei Y, Liu S, Andersson T, Feychting M, Kuja-Halkola R, Carlsson S



*Lancet Diabetes Endocrinol*. 2024;12(5):320-9.

PMID: 38561011


Brief Summary: This register-based cohort study compared the familial aggregation and heritability of childhood-onset ($\leq$18 years) *vs.* adult-onset (19-30 years) type 1 diabetes (T1D), using data collected from over 2.9 million individuals born in Sweden between 1982-2010, and from their relatives. Adult-onset T1D showed weaker familial aggregation and lower heritability than childhood-onset T1D.

Although T1D is considered a typical childhood-onset autoimmune condition, it can occur at any age. Indeed, recent data indicate that 62% of all new T1D diagnoses occur in individuals aged 20 years or older (1). Furthermore, T1D shows strong familial aggregation and high heritability (range: 0.50 to 0.88), although previous family-based and twin studies included mostly childhood-onset T1D or a mix of childhood-onset and adult-onset T1D (2).

The current study examined T1D familial aggregation and heritability by age at diagnosis. Having a first-degree relative with T1D conferred a lower risk for adult-onset T1D (hazard ratio (HR) = 7.21) than childhood-onset T1D (HR = 9.92). The HR of developing T1D at $<$ 30 years was smaller if relatives developed T1D during adulthood than during childhood. Heritability was lower for adult-onset than childhood-onset T1D (0.56 vs 0.81). These findings indicate a larger contribution of environmental factors to the development of T1D in adults than in children and highlight the importance of identifying these factors to potentially prevent T1D.

Study limitations include the upper age limit 30 years, restriction to a single country and small sample size for some types of relatives. However, it sheds light on age-differences in genetic/environmental factors in T1D pathogenesis. Further studies are needed to characterize environmental factors contributing to adult-onset T1D. Understanding these factors is crucial for developing targeted interventions to prevent T1D development and progression.

References

1. Gregory, G.A., et al., Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *Lancet Diabetes Endocrinol*. 2022;10(10):741-760.
2. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes*. 2018;19(3):346-53.

## New Mechanisms

## 10.9. Distinct cellular immune responses in children en route to type 1 diabetes with different first-appearing autoantibodies

Starskaia I, Valta M, Pietila S, Suomi T, Pahkuri S, Kalim UU, et al.
*Nat Commun*. 2024;15(1):3810.
PMID: 38714671

Brief Summary: Longitudinally collected samples from the Type 1 Diabetes Prediction and Prevention (DIPP) study were used to assess potential differences in immune responses in children at genetic risk of type 1 diabetes (T1D) who later progressed to clinical disease, stratified by autoantibody appearance (IAA-first, GADA-first, $\geq$2 autoantibodies (AAb)-first groups). Differences in the composition of peripheral blood monoclonal cells (PBMC) were found between the IAA-first, GADA-first, and $\geq$2AAb groups, highlighting the importance of endotype-specific analyses.

The prospective DIPP cohort study recruited newborns based on HLA-conferred genetic susceptibility to T1D at Turku, Oulu and Tampere University Hospitals. They were followed to age 15 years or clinical onset of T1D, with regular assessment of islet autoantibodies (1).

This DIPP analysis tested the hypothesis that children who later develop clinical T1D have different early immune responses, depending on the type of the first appearing AAb: autoantibody to insulin (IAA) vs glutamic acid decarboxylase (GADA). Previous data suggested that the rate of progression to clinical T1D is faster in IAA-first children (2).

Three groups (or endotypes) were compared, IAA-first and GADA-first children and a third group including children who tested positive for $\geq$2 AAb in the first available sample, and related controls. The composition of PBMC differed between IAA-first, GADA-first, and $\geq$2 AAb-first groups, with differences in NK cell, B cells, CD4+, CD8+ and $\gamma\delta$ TCR T cell proportions. These differences were endotype-specific and not seen when samples from all the three groups were analysed together. Differences between cases and controls were also detected in the expression of proteins in NK cells, CD4+T cells and CD8+T cells. In addition, increased expression of CD161 was detected in NK cells in children with $\geq$2 AAb compared to controls.

These findings highlight the importance of endotype-specific analyses and shed light on differences in immune responses related to T1D autoantibody, which may be useful for disease prediction and management. The study included a small number of individuals per group, and validation is needed in a larger cohort.

References

1. Ilonen, J. et al. Patterns of β-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes*. 2013;62, 3636–3640.
2. Bauer W, Veijola R, Lempainen J, Kiviniemi M, Härkönen T, Toppari J, et al. Age at Seroconversion, HLA Genotype, and Specificity of Autoantibodies in Progression of Islet Autoimmunity in Childhood. *J Clin Endocrinol Metab*. 2019;104(10):4521-30.

## 10.10. SARS-CoV-2 infection and development of islet autoimmunity in early childhood

Lugar M, Eugster A, Achenbach P, von dem Berge T, Berner R, Besser REJ, et al.
*JAMA*. 2023;330(12):1151-60.
PMID: 37682551

Brief Summary: This longitudinal cohort study of 885 infants with high genetic risk of type 1 diabetes (T1D) explored the temporal association between SARS-CoV-2 infection and development of T1D–associated autoimmunity. The incidence rate of islet autoantibodies was higher in children with vs. without SARS-CoV-2 antibodies (7.8 vs. 3.5 per 100 person-years). Hence, SARS-CoV-2 infection appears temporally associated with the development of islet autoantibodies.

Several previous studies reported an increased incidence of clinical T1D and diabetic ketoacidosis during the COVID-19 pandemic (1). This could be due to the role of viral infections as triggers for T1D autoimmunity or a direct action of SARS-CoV2 on pancreatic β-cells.

This study shifted the focus to earlier asymptomatic stages in the natural history of T1D, defined by the appearance of autoantibodies in children at genetic risk of T1D. Identifying potential modifiable targets during these early stages would allow primary prevention strategies for T1D. The study examined a unique cohort recruited in the European multicenter Primary Oral Insulin Trial (POINT), focusing on infants 4-7 months-old with a genetically defined risk of T1D $>$ 10%, originally recruited in 2018-2021 to a trial of oral insulin (2). SARS-CoV-2 infection was identified by SARS-CoV-2 antibody, and antibodies to influenza A (H1N1) were also measured.

SARS-CoV-2 antibodies developed in 170/885 children at median age 18 months (range 6-25 months), and 60 children developed islet autoantibodies. Islet autoantibodies were more likely to appear concurrent with or soon after the development of SARS-CoV-2 antibodies, but not with H1N1 antibodies. This association was strongest in children infected SARS-CoV-2 before age 18 months.

Although the findings are limited to a specific group, i.e. infants at high genetic risk of T1D, and SARS-CoV2 infection was detected by antibodies only (without PCR confirmation), these results are remarkable. These findings have prompted a vaccine trial (the Antiviral Action against Type 1 diabetes Autoimmunity (AVANT1A)) to assess whether vaccination against COVID-19 at age 6 months can prevent the development of islet autoantibodies in infants at increased genetic risk of T1D (https://www.gppad.org/de-en/avant1a/).

References

1. D'Souza, D., et al., Incidence of Diabetes in Children and Adolescents During the COVID-19 Pandemic: A Systematic Review and Meta-Analysis. *JAMA Netw Open*. 2023;6(6):e2321281.
2. Ziegler, A.G., et al., Oral insulin therapy for primary prevention of type 1 diabetes in infants with high genetic risk: the GPPAD-POInT (global platform for the prevention of autoimmune diabetes primary oral insulin trial) study protocol. *BMJ Open*. 2019;9(6):e028578.

## 10.11. Childhood-onset type 1 diabetes and subsequent adult psychiatric disorders: a nationwide cohort and genome-wide Mendelian randomization study

Formánek T, Chen D, Šumník Z, Mladá K, Hughes J, Burgess S, et al.
*Nat Ment Health*. 2024;2(9):1062-1070.
PMID: 39263363

Brief Summary: This study explored the potential causal pathways underlying the association between childhood-onset T1D and subsequent psychiatric disorders, using data from a Czech national register of 4,500 children (age $\leq$14 years) with type 1 diabetes (T1D) and large-scale European genetic studies. Children diagnosed with T1D had an elevated risk of developing substance use, mood, anxiety and personality disorders, and behavioural syndromes. In contrast, they had a lower risk of developing psychotic disorders.

Previous studies reported links between childhood-onset T1D and mental health disorders in adulthood (1,2). However, it was unclear if these associations are due to common underlying biological mechanisms or reflect the impact of managing and living with T1D. This study explored this question using a two-step approach, including observational and genetic (Mendelian randomization) analyses. Firstly, data from the Czech national register were analysed. Individuals diagnosed with T1D in childhood had a lower risk of developing psychotic disorders but a higher risk of developing most other psychiatric disorders (mood and anxiety disorders, behavioural syndromes), compared with children without T1D.

However, the Mendelian randomization analysis, using data from large-scale genome-wide association studies (GWAS) of European participants, did not support the observational findings for most studied psychiatric disorders. Only the association between T1D and lower risk of psychotic disorders or schizophrenia was consistent between observational and Mendelian randomization analyses, although the latter weakened on adjustment for multiple testing.

Overall, the findings indicate the adverse impact of living with diabetes on mental health, instead of a common underlying mechanism between T1D and psychiatric disorders. Living with T1D requires many daily tasks, including insulin injections and dose adjustments, glucose monitoring, hypo- or hyperglycemia episodes, which can lead to distress and adverse long-term mental health. This highlights the importance of supporting people with diabetes, the need for vigilance to identify early signs of distress and mental health problems, and to offer expert help when needed.

Further research is needed to explore associations between T1D and earlier-onset neurodevelopmental disorders, such as autism spectrum disorder and attention deficit hyperactivity disorder, which also tend to occur more often in young people with T1D.

References

1. Liu S, Leone M, Ludvigsson JF, Lichtenstein P, D'Onofrio B, Svensson AM, et al. Association and Familial Coaggregation of Childhood-Onset Type 1 Diabetes With Depression, Anxiety, and Stress-Related Disorders: A Population-Based Cohort Study. *Diabetes Care*. 2022;45(9):1987-93.
2. Dybdal D, Tolstrup JS, Sildorf SM, Boisen KA, Svensson J, Skovgaard AM, Teilmann GK. Increasing risk of psychiatric morbidity after childhood onset type 1 diabetes: a population-based cohort study. *Diabetologia*. 2018;61(4):831-8.

## New Biomarkers

## 10.12. A set of circulating microRNAs belonging to the 14q32 chromosome locus identifies two subgroups of individuals with recent-onset type 1 diabetes

Sebastiani G, Grieco GE, Bruttini M, Auddino S, Mori A, Toniolli M, et al.

*Cell Rep Med*. 2024;5(6):101591.
PMID: 38838677

Brief Summary: Circulating microRNAs (miRNA) were measured using multiplatform sequencing in longitudinal samples from 262 individuals with newly diagnosed type 1 diabetes (T1D) enrolled in the INNODIA Natural History Study. A set of miRNAs located within the T1D susceptibility chromosomal locus 14q32 distinguished 2 groups of T1D: those with higher miRNA expression showed better glycemic outcomes and immunomics profile.

MicroRNAs (miRNAs) are a class of small noncoding RNAs with critical roles in regulating gene expression. Previous studies suggested a role of miRNA in T1D pathogenesis by mediating function/dysfunction of pancreatic β-cells and immune cells (1). Certain miRNAs have consistently been associated with T1D onset (i.e., miR-24-3p,36–43 miR-146a-5p and miR-375-3p) or progression (i.e., miR-375-3p and miR-24-3p). Other associations were inconsistent, possibly due to variability in miRNA measurements, or differences in study populations, sample collection, or analytical platforms.

This study used 2 different sequencing platforms to measure circulating expression of miRNAs in individuals with recently diagnosed T1D, within the larger INNODIA Natural History Study cohort (2). It identified a set of

miRNAs, which defined two clusters of individuals, A and B. Three microRNAs, miR-409-3p, miR-127-3p, and miR-382-5p, which are known to be located within the T1D susceptibility chromosomal locus 14q32 (3), distinguished the two groups, with higher expression in cluster B. The identified miRNAs have several regulatory functions, including modulation of glucose metabolism. Individuals in cluster B showed better glycemic outcomes over time and higher frequency of memory and exhausted T cells.

Although the sample size was not large and replication is needed, the inclusion of a validation cohort and a well-standardized protocol for sample collection and analysis strengthens the findings (4).

These findings support the use of miR-409-3p, miR-382-5p, and/or miR-127-3p as potential biomarkers to stratify individuals with newly diagnosed T1D into two distinct subgroups, with different metabolic and immune phenotypes and potentially differing response to immunotherapies.

References


1. Margaritis K, Margioula-Siarkou G, Giza S, Kotanidou EP, Tsinopoulou VR, Christoforidis A, Galli-Tsinopoulou A. Micro-RNA Implications in Type-1 Diabetes Mellitus: A Review of Literature. *Int J Mol Sci.* 2021;22(22):12165.
2. Marcovecchio ML, Hendriks AEJ, Delfin C, Battelino T, Danne T, Evans ML, et al. The INNODIA Type 1 Diabetes Natural History Study: a European cohort of newly diagnosed children, adolescents and adults. *Diabetologia*. 2024;67(6):995-1008.
3. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. *Nature Genetics*. 2010;42(1):68-71.
4. Grieco GE, Sebastiani G, Fignani D, Brusco N, Nigi L, Formichi C, et al. Protocol to analyze circulating small non-coding RNAs by high-throughput RNA sequencing from human plasma samples. *STAR Protoc*. 2021;2(3):100606.


## 10.13. Paracrine signalling by pancreatic delta cells determines the glycaemic set point in mice


Huang JL, Pourhosseinzadeh MS, Lee S, Kramer N, Guillen JV, Cinque NH, et al.

*Nat Metab*. 2024;6(1):61-77.

PMID: 38195859


Brief Summary: This experimental study quantified the physiological contribution of pancreatic δ-cells to the glycemic set point. It used 3 orthogonal mouse models to remove somatostatin (SST) signalling within the pancreas or transplanted islets. Ablation of δ-cells or SST decreased the glycemic set point, and the glucose threshold for insulin response from β-cells, leading to increased insulin secretion to the same glucose challenge.

The roles of β- and α-cells in regulating glucose homeostasis is well defined, whereby insulin secreted by β-cells regulates glucose levels primarily in the prandial status, and glucagon produced by α-cells controls glucose levels in the fasting status (1). This study clarifies the role of the third major cell type in pancreatic islets, δ-cells, and the related production of SST, in regulating the glycemic set point and β-cell insulin production.

The study used a mouse model of inducible ablation of SST-producing cells via administration of diphtheria toxin, which ablated pancreatic δ-cells but not other SST-producing cells. This led to a persistent decrease in glucose levels and glycemic set point as well as improved glucose tolerance and plasma insulin levels. Calcium imaging, used as a proxy for insulin secretion, showed that SST changed the threshold for insulin secretion. Whereas in mice with intact δ-cells, most β-cells showed a change in calcium signalling at glucose concentrations of 8–9 mmol, in mice with ablated δ-cells, most β-cells responded at 7–8 mmol.

These findings suggest that δ-cells shift the glucose threshold for insulin secretion through local inhibitory interactions with β-cells. This δ-cell mediated mechanism may safeguard against inappropriate and acutely dangerous hyperinsulinemic hypoglycemia.

These experiments were conducted on healthy mouse models and the implications in the context of other diabetes-prone models is unclear. Further studies should clarify the potential implications in the context of type 1 or type 2 diabetes management.

References


1. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med.* 2016;48(3):e219.

## 10.14. RANKL/RANK is required for cytokine-induced beta cell death; osteoprotegerin, a RANKL inhibitor, reverses rodent type 1 diabetes

Kondegowda NG, Filipowska J, Do JS, Leon-Rivera N, Li R, Hampton R, et al.
*Sci Adv.* 2023;9(44):eadf5238.
PMID: 37910614

Brief Summary: Through a series of experiments in the nonobese diabetic/Ltj mouse model and human β-cells, the authors show that the RANK pathway mediates cytokine-induced β-cell death through RANK-TRAF6 interaction and induction of NF-KB activation. Inhibition of the RANKL/RANK pathway following treatment with osteoprotegerin and denosumab protected β-cells against this cytotoxicity, reduced proinflammatory cytokines in activated T-cells, and reversed T1D in the mouse model.

Growing evidence supports a link between glucose and bone metabolism, mediated by the key bone regulator, the receptor activator of nuclear factor-kB ligand (RANKL)/the receptor activator of NF-κB (RANK)/osteoprotegerin axis (1). RANKL and RANK are present not only in bone, but also in the liver, muscle, adipose tissue, pancreas. Previous studies showed that the RANKL/RANK pathway can inhibit β-cell replication, and inhibition of this pathway (using osteoprotegerin which binds to RANKL, and the anti-osteoporotic drug denosumab) induces rodent and human β-cell proliferation, and prevents diabetes (1,2).

This study further explored the role of RANKL/RANK in glucose regulation and immune function. A critical role of the RANKL/RANK pathway for cytokine-induced mouse and human β-cell death was identified using 3 separate approaches: i) genetic deletion of the transmembrane receptor RANK in mouse islet cells; ii) use of osteoprotegerin as a competitor of RANKL for the RANK, and iii) the RANKL antibody denosumab in human islet cells. A key intracellular pathway was identified, represented by the interaction of RANK with its intracellular signaling adaptor molecule tumor necrosis factor (TNF) receptor–associated factor 6 (TRAF6), which support the role of RANK-TRAF6 interaction in cytokine-mediated cytotoxicity. The role of the RANKL/RANK in β-cell death was confirmed by the protection of β-cells by osteoprotegerin and denosumab against cell death and dysfunction, induced by cytokines and serum from individuals with T1D. In addition, osteoprotegerin reversed recent-onset T1D in female NOD/Ltj mice. The study also confirmed the involvement of RANKL/RANK in the immune response, through the induction of inflammatory cytokines by RANKL in monocytes and reduction by OPG and DMB in activated human T cells.

These findings support the clinical and therapeutic potential of targeting the RANKL/RANK pathway in T1D, and the potential use of osteoprotegerin and/or denosumab, either alone or in combination with other immunotherapies, for the treatment of T1D.

References


1. Kondegowda NG, Fenutria R, Pollack IR, Orthofer M, Garcia-Ocaña A, Penninger JM, Vasavada RC. Osteoprotegerin and Denosumab Stimulate Human Beta Cell Proliferation through Inhibition of the Receptor Activator of NF-κB Ligand Pathway. *Cell Metab.* 2015;22(1):77-85.
2. Xing B, Yu J, Zhang H, Li Y. RANKL inhibition: a new target of treating diabetes mellitus? *Ther Adv Endocrinol Metab*. 2023;14:20420188231170754.


## 10.15. Stress-induced beta cell early senescence confers protection against type 1 diabetes

Lee H, Sahin GS, Chen CW, Sonthalia S, Canas SM, Oktay HZ, et al.
*Cell Metab.* 2023;35(12):2200-15 e9.
PMID: 37949065

Brief Summary: This experimental study unveiled a new link between the β-cell unfolded protein response and senescence. Deletion of the genes *Atf6α* or *Ire1α* in β-cells of non-obese diabetic (NOD) mice prior to insulitis generated a p21-driven early senescence phenotype, which altered the β-cell secretome and promoted protective M2 macrophages recruitment to pancreatic islets. M2 macrophages induced immune surveillance and removal of terminally senesced β cells, thus resulting in protection against type 1 diabetes (T1D).

Pancreatic β-cells are exposed to significant stress in the early stages of T1D, including endoplasmic reticulum (ER) stress (1,2). ER stress leads to the activation of the unfolded protein response (UPR), which restores homeostasis and adapts the cell to promote survival. However, in the presence of prolonged stress, UPR activates a pro-apoptotic response that results in cell death. Abnormal stress responses can also occur in the early stages of T1D, including a dysregulated UPR.

In this experimental study, the deletion of key UPR mediators, *Atf6α* or *Ire1α*, in β-cells led to an early senescence program driven by p21, resulting in a unique secretome that recruited protective immune cells, specifically M2 macrophages, to islets. M2-macrophage mediated anti-inflammatory and immunosuppressive responses along with immune surveillance, which led to reduced terminally senesced β cells, resolution of islet inflammation, reduction of β-cell apoptosis, and increased β-cell survival. Of note, the p21-mediated early senescence signature was also detected in residual β-cells of individuals with T1D.

This study highlights an early senescence programme in the restoration of islet homeostasis and attenuation of T1D, and how enhancing adaptive responses during the early stages of T1D may represent a novel approach to prevent β-cell destruction.

References

1. Yong J, Johnson JD, Arvan P, Han J, Kaufman RJ. Therapeutic opportunities for pancreatic β-cell ER stress in diabetes mellitus. *Nat Rev Endocrinol*. 2021;17(8):455-67.
2. Cha J, Aguayo-Mazzucato C, Thompson PJ. Pancreatic β-cell senescence in diabetes: mechanisms, markers and therapies. *Front Endocrinol (Lausanne)*. 2023;14:1212716.

## New Hopes

## 10.16 Non-invasive measurements of blood glucose levels by time-gating mid-infrared optoacoustic signals

Uluc N, Glasl S, Gasparin F, Yuan T, He H, Justel D, et al.
*Nat Metab*. 2024;6(4):678-86.
PMID: 38538980

Brief Summary: This study tested a new biosensor, named depth-gated mid-infrared optoacoustic sensor (DIROS), which uses intravital mid-infrared optoacoustic signals, for accurate non-invasive measurement of glucose concentrations in blood-rich volumes of the skin. DIROS provided in-blood glucose measurements with better accuracy and sensitivity than methods that measured glucose in the interstitial fluid.

Non-invasive glucose monitoring is an attractive alternative to finger pricking for blood glucose assessment (1). The introduction of continuous glucose monitoring (CGM), which uses electrochemical microneedles as a minimally invasive technique, has revolutionised the management of diabetes, making glucose monitoring easier and providing a 24-hour profile (1). However, current CGM systems measure glucose in interstitial fluids not in blood. There are discrepancies between CGM and blood glucose levels, due to differences in glucose dynamics in these compartments, which potentially result in inaccurate measurements.

This study tested in mice a new biosensor, termed depth-gated mid-infrared optoacoustic sensor (DIROS), which allows non-invasive glucose detection in blood-rich volumes in the skin. This system can detect glucose levels directly from the blood, using optoacoustic signals from the mid-infrared spectrum. DIROS sends pulses of light into the tissue, which converts the light into ultrasound waves that can be detected on the skin surface. It uses time-gated optoacoustic measurements, which can remove the contributions from tissue components on the skin surface that might reduce measurement accuracy.

This new technology represents a remarkable step towards the development of non-invasive glucose measuring devices. Next step is to assess the performance of DIROS at different areas of the human skin and to translate these findings into clinical practice as an alternative way of monitoring glucose levels in people living with diabetes.

References

1. Di Filippo D, Sunstrum FN, Khan JU, Welsh AW. Non-Invasive Glucose Sensing Technologies and Products: A Comprehensive Review for Researchers and Clinicians. *Sensors*. 2023;23(22):9130.

## 10.17. Non-invasive quantification of stem cell-derived islet graft size and composition

Lithovius V, Lahdenpohja S, Ibrahim H, Saarimaki-Vire J, Uusitalo L, Montaser H, et al.

*Diabetologia*.

2024;67(9):1912-29. PMID: 38871836

Brief Summary: This experimental study used positron emission tomography (PET) as non-invasive method to monitor stem cells-islet grafts. [$^{18}$F]exendin and [$^{18}$F]FDOPA PET showed potential as non-invasive methods to assess stem cells-islet graft size and aspects of graft composition.

Stem cell-derived islets (SC-islets) are used as an emerging cell replacement therapy for insulin-dependent diabetes (1). Non-invasive long-term monitoring methods for SC-islet grafts are needed to detect potential unwanted expansion and to optimise their effectiveness.

PET has previously been used to monitor transplanted primary islets. Here it was tested as non-invasive way to monitor SC-islet grafts in mice. Different doses of human SC-islets were implanted and the grafts were then monitored with PET using two tracers, glucagon-like peptide 1 receptor-binding [$^{18}$F]F-dibenzocyclooctyne-exendin-4 ([$^{18}$F]exendin) and the dopamine precursor 6-[$^{18}$F]fluoro-l-3,4-dihydroxyphenylalanine ([$^{18}$F]FDOPA), for 5 months, followed by histological assessment of graft size and composition.

The study showed a successful application of [$^{18}$F]exendin and [$^{18}$F]FDOPA PET, which allowed accurate quantification of total SC-islet graft volume. In addition, [$^{18}$F]exendin PET enabled assessment of SC-islet graft quality by providing information about graft stem cell-derived β-cell and cyst composition.

These findings are promising, but they need to be replicated and tested in humans. Nevertheless, the study brings hopes that PET imaging might become a tool to improve the safety and efficacy of SC-islet grafts as an emerging cell replacement therapy for type 1 diabetes.

References

1. Hogrebe NJ, Ishahak M, Millman JR. Developments in stem cell-derived islet replacement therapy for treating type 1 diabetes. *Cell Stem Cell*. 2023;30(5):530-48.

# 11. Obesity and Weight Regulation

Martin Wabitsch, Stephanie Brandt-Heunemann, Stefanie Zorn, Christian Denzer, Melanie Schirmer, Nicole Prinz, Joanna Lerner, Eleni Giannopoulou, Daniel Tews
Division of Pediatric Endocrinology and Diabetes and Endocrine Research Laboratory, Department of Pediatrics and Adolescent Medicine, University of Ulm, Ulm, Germany

## Preface

As in previous years in this Yearbook 2024 chapter we can only present 1.5 % of the acquired publications (1,656) according to our search criteria in PubMed. The last year has again been extremely exciting for the field of obesity and weight regulation and it was a significant step into the future in terms of scientific output.

Of particular importance is the new pharmacological therapeutic approach for patients with acquired hypothalamic obesity (see Roth et al., *Lancet Diabetes Endocrinol* 2024 Jun;12(6):380-389. doi:10.1038/s41591-022-01902-3). For this group of patients, in whom extreme obesity is often the main burden of disease, a non-surgical treatment now appears to exist that for the first time can achieve clinically relevant weight reduction.

The Yearbook chapter 2024 on obesity and weight regulation comprises further exciting articles covering a broad research area.

## Interventions for Weight Loss: New Findings

### 11.1. Height and growth velocity in children and adolescents undergoing obesity treatment: a prospective cohort study

Putri RR, Danielsson P, Marcus C, Hagman E
Division of Pediatrics, Department of Clinical Science, Intervention, and Technology, Karolinska Institutet, Stockholm 141 57, Sweden.
*J Clin Endocrinol Metab*. 2023 Dec 21;109(1):e314-e320.
doi:10.1210/clinem/dgad419.
https://pubmed.ncbi.nlm.nih.gov/37453086/

Brief summary: This large cohort study of 27,997 individuals aged 3-18 years with obesity in Sweden examined the effect of obesity severity and obesity treatment on growth. Prepubertal individuals with class II (more severe) obesity were had higher height-for age and growth velocity than children with class I obesity. After onset of puberty, these individuals and especially boys, showed slower height velocity and a blunted growth spurt. The final height z score in this study population was slightly lower than in the Swedish reference population. Successful obesity treatment "normalized" the accelerated growth velocity pattern in children with obesity.

These results provide important understanding into how childhood obesity impacts on growth. Childhood BMI is an important modifier of childhood and pubertal growth (1-4). Advanced early linear growth in children with obesity (2, 3) has been associated with negative cardiovascular health outcomes (4). Healthcare providers managing children with obesity should be aware of these distinct growth patterns in order to prevent them from unnecessary examinations. In other cases, a different than expected growth pattern could suggestive another etiology (syndromic or endocrine disorder). These authors suggest that a height z score $< 0.0$ in children age $<$ 10 years with obesity may indicate growth impairment that needs further evaluation. Previously reported height reference values for children with obesity allow for appropriate assessments (2).

Large prospective and longitudinal studies in real-world settings assessing how obesity treatment outcome affect long-term growth are lacking. These data show for the first time that a successful obesity treatment (defined as BMI $z$ score reduction $\leq 0.25$ units after 1-year) could "normalize" the growth pattern of children with obesity. The cutoff of 0.25 units was chosen because a BMI $z$ score reduction of $0.25+$ units is associated with improvements in obesity-related consequences in previous research (5, 6). Further research on longitudinal data of growth after obesity treatment in children is needed to confirm this clinically important finding.


References

1. Holmgren A, Martos-Moreno G, Niklasson A, Martínez-Villanueva J, Argente J, Albertsson-Wikland K. The pubertal growth spurt is diminished in children with severe obesity. *Pediatr Res.* 2021;90(1):184-90.
2. Kempf E, Vogel M, Vogel T, Kratzsch J, Landgraf K, Kühnapfel A, et al. Dynamic alterations in linear growth and endocrine parameters in children with obesity and height reference values. *EClinicalMedicine*. 2021;37:100977.
3. He Q, Karlberg J. Bmi in childhood and its association with height gain, timing of puberty, and final height. *Pediatr Res.* 2001;49(2):244-51.
4. Antonisamy B, Vasan SK, Geethanjali FS, Gowri M, Hepsy YS, Richard J, et al. Weight Gain and Height Growth during Infancy, Childhood, and Adolescence as Predictors of Adult Cardiovascular Risk. *J Pediatr.* 2017;180:53-61.e3.
5. Reinehr T, Kleber M, Toschke AM. Lifestyle intervention in obese children is associated with a decrease of the metabolic syndrome prevalence. *Atherosclerosis*. 2009;207(1):174-80.
6. Putri RR, Casswall T, Hagman E. Risk and protective factors of non-alcoholic fatty liver disease in paediatric obesity: A nationwide nested case-control study. *Clin Obes.* 2022;12(2):e12502.


## 11.2. Mutations in the leptin-melanocortin pathway and weight loss after bariatric surgery: a systematic review and meta-analysis


Zhang N, Wang H, Ran S, Wang Z, Zhou B, Wang S, Li Z, Liu B, Nie Y, Huang Y, Meng H

General Surgery Department & Obesity and Metabolic Disease Center, China-Japan Friendship Hospital, Beijing, China.



*Obesity (Silver Spring).* 2024 Jun;32(6):1047-1058.

doi:10.1002/oby.24007.

https://pubmed.ncbi.nlm.nih.gov/38577709/


Brief summary: This review evaluated the impact of gene mutations in the leptin-melanocortin pathway on short- and long-term outcomes after bariatric surgery, which is still the most effective treatment for severe obesity. This topic is of high clinical relevance, since such mutations play an important role in hunger regulation and energy homeostasis [1, 2] and may therefore confer weight regain after bariatric surgery [3]. To date there have been few publications, often with only small numbers of cases, and some with contradictory results [4, 5, 6].

This meta-analysis identified 7 publications from 2003 to 2023, including 13 datasets with a total of 301 mutation carriers (n = 182 with MC4R mutations and n = 119 with mutations in the whole pathway) and 1966 control patients. Meta-regression was used to quantify the effect of mutations on short-term ($<12$ months) and long-term (2– 6 years) weight loss after bariatric intervention. Patients with mutations in the leptin-melanocortin pathway achieved less total weight loss after bariatric surgery (mean difference between groups: -3.03%; 95% CI: -3.63 to -2.44), which was primarily evident in reduced long-term (2-6 years) postoperative weight loss (-3.43%; 95% CI: -4.09 to -2.77). By contrast, up to 12 months after surgery, mutation carriers showed similar weight loss than those without mutations (-1.13%; 95% CI: -2.57 to 0.31).

Due to the still limited number of cases and non-existent information, it was not possible to examine differences in outcomes based on genetic subgroups, surgical procedures or patient baseline conditions. Large international cohort studies with detailed phenotyping are needed to investigate the influence of genetic and other factors in more detail. However, this review makes an important contribution to the understanding of genetic factors influencing bariatric outcomes, by showing a lower but still distinct weight loss in mutations carriers after bariatric surgery. Against the average ~25-30% weight loss after bariatric intervention [7-8], a difference of only 3% indicates that a mutation in the leptin-melanocortin pathway is not a contraindication for bariatric surgery.

Genetic screenings could predict weight loss outcome after bariatric surgery, which might help tailor surgical procedures to individual patients, optimize outcome by additional intervention such as e.g. medication and thus improving long-term success rates.


References

1. Loos RJF Yeo GSH. The genetics of obesity: from discovery to biology. *Nat. Rev Genet.* 2022; 23(2): 120-133
2. Farooqi S, O'Rahilly S. Genetics of obesity in humans. *Endocr. Rev.* 2006; 27:710–718. doi:10.1210/er.2006-0040.

3. Hatoum IJ, Stylopoulous N, Vanhoose AM et al. Melanocortin-4 receptor signaling is required for weight loss after gastric bypass surgery. *J Clin Endocrinol Metab.* 2012; 97(6):E1023-1031
4. Mirshahi UL, Still CD, Msker KK et al. The MC4R(I251L) allele is associated with better metabolic status and more weight loss…. *J Clin Endocrinol Metab.* 2011;96(12):E2088-E2096
5. Valette M, Poitou C, Le Beyec J, et al. Melanocortin-4 receptor mutations and polymorphisms do not affect weight loss after bariatric surgery. *PloS One.* 2012; 7(11):e48221
6. Cooiman MI, Alsters SIM, Duquesnoy M, et al. Long-term weight outcome after bariatric surgery… *Obes Surg.* 2022;32(3):837-844
7. Inge TH. Five-Year Outcomes of Gastric Bypass in Adolescents as Compared with Adults. *N Engl J Med.* 2019 May 30; 380(22): 2136–2145. doi:10.1056/NEJMoa1813909.
8. Courcoulas AP et al. Seven-Year Weight Trajectories and Health Outcomes in the Longitudinal Assessment of Bariatric Surgery (LABS) Study. *JAMA JAMA Surg.* 2018 May; 153(5): 427–434.

## 11.3. Who benefits most from outpatient lifestyle intervention? an IMI-SOPHIA study on pediatric individuals living with overweight and obesity

Prinz N, Pomares-Millan H, Dannemann A, Giordano GN, Joisten C, Körner A, Weghuber D, Weihrauch-Blüher S, Wiegand S, Holl RW, Lanzinger S
Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany.
*Obesity (Silver Spring)*. 2023 Sep;31(9):2375-2385.
doi:10.1002/oby.23844.
https://pubmed.ncbi.nlm.nih.gov/37545199/

Brief summary: For both clinicians and patients, it is of great interest to have easily-applicable predictors to forecast weight reductions achievable by lifestyle intervention (LI). This study identified 3 distinct clusters of responses to paediatric outpatient LI. Up to 2 years after LI initiation, responses ranged from 'pronounced BMIz loss' (in 19% of patients; median delta-BMIz: -0.61 (IQR: -0.76 to -0.49) to 'moderate BMIz loss' (in 45%; -0.23 (-0.33 to -0.14)) and 'no BMIz loss' (or even an BMIz increase) (in 36%). Greater LI response was predicted by younger age (5 to $\leq$11 years), lower baseline BMIz, greater initial BMIz reduction (at least 5%), and longer LI duration ($\geq$12 months). By contrast, living in socioeconomically deprived areas and having a family history of obesity predicted limited response to LI.

This study highlights again the need for early, and sustained, personalized intervention among pediatric individuals living with overweight and obesity. In high-risk subjects with likely limited benefit from LI, early treatment escalation using approved weight-reduction drugs or bariatric surgery in addition to LI might be considered to support weight loss. However, large heterogeneity to semaglutide treatment in addition to LI among adolescents ($>$12 years) has been reported and warrants better understanding of the underlying mechanisms [1]. Additional studies on the economic benefits of timely, patient-tailored intervention and prevention programs might help to convince health care insurances and policy makers in the future. LI programs are often characterized by high drop-out rates. Thereby, the current study may be biased due to the inclusion criteria of multiple follow-up visits, so only families with good adherence to LI might have been included. This in turn may explain the high success rate; $>$60% of individuals achieved BMIz reduction $\geq$0.2 within 2 years. As the study is from Germany, its generalizability to other ethnic groups or countries with different healthcare systems is unclear.

Reference

1. Weghuber, D.; Barett T.; Barrientos-Pérez, M.; Gies, I.; Hesse D.; Jeppesen, O.K.; Kelly, A.S.; Mastrandrea, L.D.; Sørrig, R.; Arslanian, S.; Once-Weekly Semaglutide in Adolescents with Obesity. *N Engl J Med* 2022, 387 (24), 2245-2257. doi:10.1056/NEJMoa2208601.

## 11.4. Setmelanotide for the treatment of acquired hypothalamic obesity: a phase 2, open-label, multicentre trial

Roth CL, Scimia C, Shoemaker AH, Gottschalk M, Miller J, Yuan G, Malhotra S, Abuzzahab MJ
Seattle Children's Research Institute, Seattle, WA 98101, USA.
christian.roth@seattlechildrens.org
*Lancet Diabetes Endocrinol* 2024 Jun;12(6):380-389.
doi:10.1038/s41591-022-01902-3.
https://pubmed.ncbi.nlm.nih.gov/35879615/

Brief summary: This phase 2, open-label, multicenter trial examined the efficacy of setmelanotide, a melanocortin-4 receptor agonist, in patients aged 6 to 40 years with obesity due to hypothalamic injury. Setmelanotide reduced body mass index (BMI) and hunger levels in most participants; 89% achieved a reduction in BMI of at least 5% after 16 weeks. These results indicate that setmelanotide is a promising treatment for hypothalamic obesity.

This phase 2 trial on setmelanotide for acquired hypothalamic obesity marks a pivotal advancement in addressing a historically intractable condition. Hypothalamic obesity often follows treatment for craniopharyngioma, and has been linked to severe metabolic dysregulation and an increased risk of premature mortality (1). Thus far, a variety of interventions, including dextro-amphetamine (2, 3), somatostatin analoges (4) and GLP- 1R agonists, were only partially successful or yielded inconsistent outcomes (5)?. Setmelanotide, a melanocortin-4 receptor (MC4R) agonist, has shown promise in treating monogenic obesity due to mutations in the leptin-melanocortin pathway? (6). The current trial found a significant reduction in BMI and hunger levels in patients with hypothalamic obesity. Therefore, setmelanotide could potentially address the disrupted melanocortin signaling due to hypothalamic damage.

However, the heterogeneity of hypothalamic obesity—rooted in varying degrees of hypothalamic injury—presents challenges in standardizing treatment outcomes. These encouraging trial results indicate that even in non-homogeneous patient populations, targeting the melanocortin pathway might yield substantial benefits. Still, the necessity for a double-blind, randomized phase 3 trial cannot be overstated to confirm these findings.

Moreover, the conceptualization of setmelanotide as a form of hypothalamic substitution therapy, rather than merely an anti-obesity drug, warrants further exploration. If proven effective in larger trials, setmelanotide could redefine the therapeutic landscape for hypothalamic obesity, offering a novel, precision-based approach to managing this complex condition.

References


1. Müller HL, Tauber, M, Lawson, EA, Özyurt, J, Bison, B, Martinez-Barbera, JP et al. *Nat Rev Dis Prim* (2022) 8:24. doi:10.1038/s41572-022-00351-z.
2. Denzer C, Denzer F, Lennerz BS, Vollbach H, Lustig RH, Wabitsch M. Treatment of hypothalamic obesity with dextroamphetamine: A case series. *Obes Facts* (2019) 12:91-102. doi:10.1159/000495851.
3. van Schaik J, Welling MS, de Groot CJ, van Eck JP, Juriaans A, Burghard M, et al. Dextroamphetamine treatment in children with hypothalamic obesity. *Front Endocrinol* (2022) 13:845937. doi:https://doi.org/10.3389/fendo.2022.845937.
4. Lustig RH, Rose SR, Burghen GA, Velasquez-Mieyer P, Broome DC, Smith K, et al. Hypothalamic obesity caused by cranial insult in children: altered glucose and insulin dynamics and reversal by a somatostatin agonist. *J Pediatr* (1999) 135:162-8. doi:10.1016/S0022-3476(99)70017-X.
5. van Iersel L, Brokke KE, Adan RAH, Bulthuis LCM, van den Akker ELT, van Santen HM. Pathophysiology and individualized treatment of hypothalamic obesity following craniopharyngioma and other suprasellar tumors: A systematic review. *Endocr. Rev* (2018) 40:193-235. doi:10.1210/er.2018-00017.
6. Clément K, van den Akker E, Argente J, Bahm A, Chung WK, Connors H, et al. Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials. *Lancet Diabetes Endocrinol* (2020) 8:960-70. doi:10.1016/S2213-8587(20)30364-8.


## 11.5. Tirzepatide modulates the regulation of adipocyte nutrient metabolism through long-acting activation of the GIP receptor


Regmi A, Aihara E, Christe ME, Varga G, Beyer TP, Ruan X, Beebe E, O'Farrell LS, Bellinger MA, Austin AK, Lin Y, Hu H, Konkol DL, Wojnicki S, Holland AK, Friedrich JL, Brown RA, Estelle AS, Badger HS, Gaidosh GS, Kooijman S, Rensen PCN, Coskun T, Thomas MK, Roell W

Eli Lilly and Company, Indianapolis, IN 46285, USA. bill_roell@lilly.com



*Cell Metab* 2024 36(7): 1534-1549.e7.

doi:10.1016/j.cmet.2024.05.010.

https://pubmed.ncbi.nlm.nih.gov/38878772.


Brief summary: The study investigated how tirzepatide, a dual GIPR/GLP-1R agonist, modulates adipocyte nutrient metabolism by long-acting activation of the GIP receptor. It shows that tirzepatide enhances insulin signaling, glucose uptake, and lipid metabolism in adipocytes, contributing to improved metabolic outcomes in both fasted and fed states.

Tirzepatide has recently been shown to have superior efficacy in reducing HbA1c, body weight, and serum triglycerides compared to selective GLP-1R agonists[1]. Prior studies demonstrated that GLP1R is not primarily expressed on adipocytes. The current study provides substantial evidence of GIPR expression and functional activation in adipocytes, highlighting a significant role in adipocyte metabolism regulation. The regulation of key metabolic transcription factors and pathways by tirzepatide aligns with existing knowledge on the importance of these pathways in maintaining metabolic homeostasis. These findings add depth by demonstrating the specific contributions of GIPR agonism and advance the understanding of tirzepatide's role in adipocyte nutrient metabolism and its superior clinical efficacy. By elucidating the dual mechanisms of GIPR and GLP-1R agonism, this research provides a robust framework for the development of more effective treatments for T2D and obesity.

Reference

1. Frías, J. P., Davies, M. J., Rosenstock, J., Pérez, Manghi, F. C., Fernández, Landó, L, Bergman, B. K., Liu, B., Cui,, X., & Brown K. (2021). Tirzepatide, versus Semaglutide Once Weekly in Patients with Type 2 Diabetes. *New England Journal of Medicine*, 385(6), 503–515. https://doi.org/10.1056/nejmoa2107519.

## History, Language and Numbers

## 11.6. Obesity: a 100 year perspective

Bray GA

Pennington Biomedical Research Center/LSU, Baton Rouge, LA, 70808, USA. george.bray@pbrc.edu.

*Int J Obes (Lond)*. 2024 May 7.

doi:10.1038/s41366-024-01530-6. Epub ahead of print. PMID: 38714830.

https://pubmed.ncbi.nlm.nih.gov/38714830/

Brief summary: This review describes how views and understanding of obesity have changed over the last 100 years and how new therapies have been developed. The article summarizes key milestones in knowledge gains that led to the science community's current understanding. It also shows how the scientific world has dealt with this gain in knowledge in terms of the activity of professional societies and scientific publications. The review closes with a few important aphorisms about obesity learned over the past.

The author Dr George Bray has dedicated his professional life to the research in obesity and metabolism. He has gained international recognition for his pioneering research on both the experimental and clinical levels. With his perspective and personal scientific experience over more than 60 years, he is one of the few people who can reflect the historical development to a large extent as a witness.

The assessment of obesity with regard to its development depended on the available scientific methods. The evaluation of large epidemiological data sets in combination with genetic analyses impressively demonstrated the strong dependence of body fat mass on genetic predisposition and the living environment. The study of rare syndromic obesity forms in mice and humans have paved the way for the definition of subtypes and the development of effective pharmacological treatments. Mythical beginnings, errors and corrections in the understanding of energy metabolism and the resulting attempts at intervention have led to today's recognition of obesity as a chronic relapsing progressive disease characterized by an inflammatory state and associated with significant mortality and morbidity (1). There is still a long way to go for these findings to save affected patients from painful odysseys and to dispel the obesity stigma in society (2).

References

1. Bray GA, Kim KK, Wilding JPH; World Obesity Federation. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. *Obes Rev.* 2017 Jul;18(7):715-723. doi:10.1111/obr.12551. Epub 2017 May 10. PMID: 28489290.
2. Rubino F, Puhl RM, Cummings DE, Eckel RH, Ryan DH, Mechanick JI, Nadglowski J, Ramos Salas X, Schauer PR, Twenefour D, Apovian CM, Aronne LJ, Batterham RL, Berthoud HR, Boza C, Busetto L, Dicker D, De Groot M, Eisenberg D, Flint SW, Huang TT, Kaplan LM, Kirwan JP, Korner J, Kyle TK, Laferrère B, le Roux CW, McIver L, Mingrone G, Nece P, Reid TJ, Rogers AM, Rosenbaum M, Seeley RJ, Torres AJ, Dixon JB. Joint international consensus statement for ending stigma of obesity. *Nat Med.* 2020 Apr;26(4):485-497. doi:10.1038/s41591-020-0803-x. Epub 2020 Mar 4. PMID: 32127716; PMCID: PMC7154011.

## 11.7. Providing a common language for obesity: the European Association for the Study of Obesity obesity taxonomy

Bowman-Busato J, Schreurs L, Jason H, Yumuk V, O'Malley G, Woodward E, De Cock D, Baker JL
Biostatistics and Medical Informatics Research Group, Department of Public Health, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel (VUB), 1090, Brussels, Belgium.
lucas.schreurs@vub.be.
*International Journal of Obesity.* 2024 Jun 20; Online ahead of print.
doi:10.1038/s41366-024-01565-9.
https://pubmed.ncbi.nlm.nih.gov/38902385/

Brief summary: The European Association for the Study of Obesity (EASO) initiated this online Delphi study. They invited an expert panel of n = 194 stakeholders, including policymakers, healthcare professionals, people living with obesity, and researchers from 30 countries to evaluate proposed statements on obesity to create a standardised language. Based on the understanding of obesity as an adiposity-based chronic disease, consensus was achieved on 54 statements categorized into 6 themes: (1) Definition, (2) Causes, onset and progression, (3) Prevention, (4) Screening and diagnosis, (5) Treatment and management, (6) Consequences.

Establishing a common, precise, and scientifically accurate language for obesity is essential for effective communication and to build mutual understanding of obesity as a chronic disease, from primary prevention to diagnosis and treatment. People with obesity, especially children, often face weight bias from healthcare staff, colleagues, teachers, peers, and their family. This leads to serious life-long consequences, such as poor mental health, poor quality of life, and poor social, academic and weight management outcomes [1]. Although the use of person-first language to end weight bias and stigma is increasing in scientific publications on obesity [2], obesity is often misconceived by the general population as a result of "lack of individual willpower" and the responsibility of the individual, ignoring the scientific evidence that obesity is a neuro-metabolic disease with complex biological, genetic, psychosocial and environmental drivers [3].

This taxonomy is an important first step in bridging the gap between scientific knowledge and conventional narratives around obesity. It aims to increase understanding of obesity, improve education on the physiological basis and clinical management, and facilitate a clear communication. To maximise its impact, this taxonomy needs to be widely adopted and implemented in the communication between health care professionals, policymakers, researchers, and people living with obesity.

References

1. Puhl, R.M.; Lessard, L.M.; Weight Stigma in Youth: Prevalence, Consequences, and Considerations for Clinical Practice. *Curr Obes Rep.* 2020 Dec;9(4):402-411. doi:10.1007/s13679-020-00408-8.
2. Weghuber, D.; Khandpur, N.; Boyland, E.; Mazur, A.; Frelut, M.L.; Forslund, A.; Vlachopapadopoulou, E.; Erhardt, É.; Vania, A.; Molnar, D.; Ring-Dimitriou, S.; Caroli, M.; Mooney, V.; Forhan, M.; Ramos-Salas, X.; Pulungan, A.; Holms, J.C.; O'Malley, G.; Baker, J.L.; Jastreboff, A.M.; Baur, L.; Thivel, D. Championing the use of people-first language in childhood overweight and obesity to address weight bias and stigma: A joint statement from the European-Childhood-Obesity-Group (ECOG), the European-Coalition-for-People-Living-with-Obesity (ECPO), the International-Paediatric-Association (IPA), Obesity-Canada, the European-Association-for-the-Study-of-Obesity Childhood-Obesity-Task-Force (EASO-COTF), Obesity Action Coalition (OAC), The Obesity Society (TOS) and the World-Obesity-Federation (WOF). *Pediatr Obes.* 2023 Jun;18(6):e13024. doi:10.1111/ijpo.13024.
3. O'Keeffe, M.; Flint, S.W.; Watts, K.; Rubino F. Knowledge gaps and weight stigma shape attitudes toward obesity. *Lancet Diabetes Endocrinol.* 2020 May;8(5):363-365. doi:10.1016/S2213-8587(20)30073-5.

## 11.8. Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults

NCD Risk Factor Collaboration (NCD-RisC)
*Lancet*. 2024 Mar;403(10431):1027-1050.
doi:10.1016/S0140-6736(23)02750-2.
https://pubmed.ncbi.nlm.nih.gov/38432237/

Brief summary: Global data are still scarce on the combined burden of obesity and underweight, and its changes over time across countries and age groups. The previous prevalences of obesity and underweight were published in 2016 [1]. This analysis pooled representative, world-wide samples of the general population, including 3,663 studies with 222 million participants aged 5 years and older with weight and height measurements to estimate

the combined and individual prevalence of underweight and obesity between 1990 to 2022. It used the WHO growth reference as standard for pediatrics (63 million participants aged 5-19 years).

From 1990 to 2022, the combined prevalence of obesity and underweight increased in most countries, mainly driven by a shift from underweight dominance to obesity dominance. This shift was largest in South Africa for girls and in China for boys. This shift was already seen in adults in 1990, and has now reached school-aged children and adolescents [2]. The global age standardized prevalence of pediatric obesity increased 4-fold, while those of pediatric thinness decreased more slowly. Some low-income and middle-income countries now have a higher obesity prevalence than industrialized, high-income countries.

Despite some differences in data quality and coverage across regions, this is an excellent-designed world-wide analysis indicating alarming rates of obesity in childhood and adulthood worldwide. Aside from effective, sustainable treatment programs, a major challenge is to reduce disease risk through primary prevention on all levels starting even in the youngest groups. This should involve improved access to affordable healthy foods, promoting healthy dietary habits/physical activity and implementing comprehensive, effective public health policies. To what extent public health strategies such as taxation on sugary drinks or restrictions on unhealthy food marketing, already implemented in few countries, impact obesity rates is currently evaluated [3]. Aside from obesity, persistent underweight in some countries endorse efforts to improve access to nutritious foods and addressing underlying socio-economic factors.

References


1. NCD Risk Factor Collaboration (NCD-RisC); Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128-9 million children, adolescents, and adults. *Lancet* 2017, 390, 2627-2642. doi:10.1016/S0140-6736(17)32129-3.
2. Jaacks, L.M.; Vandevijvere, S.; Pan, A.; McGowan, C.J.; Wallace, C.; Imamura, F.; Mozaffarian, D.; Swinburn, B.; Ezzati, M.; The obesity transition: stages of the global epidemic. *Lancet Diabetes Endocrinol* 2019, 7, 231–240. doi:10.1016/S2213-8587(19)30026-9.
3. Taillie, L.S.; Busey, E., Stoltze, F.M.; Dillman Carpentier, F.R; Governmental policies to reduce unhealthy food marketing to children. *Nutr Rev* 2019, 77, 787–816. doi:10.1093/nutrit/nuz021.


## Genetic Risk Score and New Genes

### 11.9. Elucidating pathways to pediatric obesity: a study evaluating obesity polygenic risk scores related to appetitive traits in children


Renier TJ, Yeum D, Emond JA, Lansigan RK, Ballarino GA, Carlson DD, Loos RJF, Gilbert-Diamond D
Department of Epidemiology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA.
Timothy.J.Renier.GR@ dartmouth.edu.



*International Journal of Obesity.* 2024 Jan; 48(1):71-77.
doi:10.1038/s41366-023-01385-3.
https://pubmed.ncbi.nlm.nih.gov/37736781/


Brief summary: This genetic epidemiology study genotyped n = 248 unrelated children aged 9-12 years, computed 4 weighted polygenic risk scores (PRS), and investigated the associations between (1) appetitive traits and BMI, (2) PRSs and BMI, and (3) PRSs and appetitive traits. Appetite traits were evaluated using an obesogenic appetite score, which was calculated by combining mean scores of the food approach and food avoidance domains from the Child Eating Behavior Questionnaire. There were positive associations between (1) appetitive traits and BMI, (2) between all 4 PRSs and BMI, and (3) between 3 PRSs and appetitive traits. Notably, a significant partial mediation of the PRS-BMI association by the obesogenic appetite score was identified for 3 PRSs, accounting for 11.3% and 21.3% of the total association, depending on the PRS used.

A previous cross-sectional survey of n = 5275 adolescents with obesity identified the inability to control appetite and hunger as the main barrier to successful weight reduction [1]. Insights from patients with rare forms of monogenic obesity have underscored the profound impact of genetic variants in the regulation of hunger and satiety in the hypothalamus, leading to weight gain when these processes are disrupted [2]. However, these rare

genetic variants are typically found in individual cases, suggesting that "common" obesity results from complex interactions between genetic susceptibility and exposure to an obesogenic environment. The Behavioural Susceptibility Theory offers a possible explanation for the development of "common" obesity in children. It posits that genetic factors can lead to variations in appetite and, in combination with the availability of higher palatable food, e.g. in an obesogenic environment, promote unfavourable eating behaviour and increase energy intake relative to energy expenditure and consequently weight [2, 3].

These findings support the Behavioural Susceptibility Theory by providing evidence for gene-appetite-environment interactions and contributes to our understanding of the underlying mechanism of obesity. However, further research with larger populations is needed to generalize the findings and to develop appropriate implications for policy and individual lifestyle interventions.

References

1. Halford, J.C.G.; Bereket, A.; Bin-Abbas, B.; Chen, W.; Fernández-Aranda, F.; Garibay Nieto, N.; López Siguero, J.P.; Maffeis, C.; Mooney, V.; Osorto, C.K.; Reynoso, R.; Rhie, Y.J.; Toro-Ramos, M.; Baur, L.A. Misalignment among adolescents living with obesity, caregivers, and healthcare professionals: ACTION Teens global survey study. *Pediatr Obes.* 2022 Nov;17(11):e12957. doi:10.1111/ijpo.12957.
2. Loos, R.J.F.; Yeo, G.S.H. The genetics of obesity: from discovery to biology. *Nat Rev Genet* 23, 120–133 (2022). https://doi.org/10.1038/s41576-021-00414-z.
3. Llewellyn, C.; Wardle, J. Behavioral susceptibility to obesity: Gene-environment interplay in the development of weight. *Physiol Behav.* 2015 Dec 1;152(Pt B):494-501. doi:10.1016/j.physbeh.2015.07.006.
4. Llewellyn, C.H.; Kininmonth, A.R.; Herle, M.; Nas, Z.; Smith, A.D.; Carnell, S.; Fildes, A. Behavioural susceptibility theory: the role of appetite in genetic susceptibility to obesity in early life. *Philos Trans R Soc Lond B Biol Sci.* 2023 Sep 11;378(1885):20220223. doi:10.1098/rstb.2022.0223.

## 11.10. Protein-truncating variants in BSN are associated with severe adult-onset obesity, type 2 diabetes and fatty liver disease

Zhao Y, Chukanova M, Kentistou KA, Fairhurst-Hunter Z, Siegert AM, Jia RY, Dowsett GKC, Gardner EJ, Lawler K, Day FR, Kaisinger LR, Tung YCL, Yee Hong Lam B, Chen HJC, Wang Q, Berumen-Campos J, Kuri-Morales P, Tapia-Conyer R, Alegre-Diaz J, Barroso I, Emberson J, Torres JM, Collins R, Saleheen D, Smith KR, Paul DS, Merkle F, Farooqi IS, Wareham NJ, Petrovski S, O'Rahilly S, Ong KK, Yeo GSH, Perry JRB

MRC Epidemiology Unit and NIHR Cambridge Biomedical Research Centre, Institute of Metabolic Science, University of Cambridge, School of Clinical Medicine, Cambridge, UK.

John.Perry@mrc-epid.cam.ac.uk

*Nat Genet* 56, 579–584 (2024).
https://doi.org/10.1038/s41588-024-01694-x,
https://pubmed.ncbi.nlm.nih.gov/38575728/

Brief summary: This exome-wide association study conducted in the UK Biobank cohort (n = 454 787) and in two non-European cohorts, the Mexican MCPS cohort (n = 141 046) and the Pakistani PGR cohort (n = 37 800), identified a association between rare protein-truncating variants (PTVs) in the *APBA1* and the *BSN* genes and adult-onset obesity, suggesting two new genes as possible causes for monogenic obesity. Rare PTVs in *BSN* were also associated with Type 2 diabetes and fatty liver disease. Screening for rare PTVs in *APBA1* and *BSN* in the Severe Childhood-Onset Obesity Project (SCOOP) cohort (n = 927) detected 3 individuals with rare PTVs in *BSN*.

Identification of genes and genetic variants as possible causes for monogenic obesity is important for gaining new insights into weight regulation pathways and consequently new therapeutic strategies. In contrast to previously described forms of monogenic obesity, characterized by early-childhood obesity, this study examined genetic variants causing adult-onset obesity in 3 populations of different ethnicity. *APBA1* is involved in signal transduction in the central nervous system [1] and in glucose homeostasis [2,3]. *In vitro* experiments in stem cell-derived hypothalamic neurons with PTVs in *BSN* revealed a reduced expression of *SEMA3C* which causes obesity in animal models through interfering with the leptin-melanocortin pathway [4]. Thus, PTVs in these genes could potentially cause obesity before adulthood, as shown by the appearance of rare PTVs in *BSN* in the SCOOP cohort. In a separate paper, Zhu et al. reported 2 cases with rare loss-of-function variants in *BSN* who had obesity in teenage years [5], suggesting that variants in *BSN* may cause obesity before reaching adulthood. Taken together, these considerations support the suspicion that rare PTVs in *BSN* and *APBA1* are associated with adult-onset obesity but may be relevant for early-onset obesity and should be included in diagnostic panel testing for monogenic forms of obesity in both children and adults.

References

1. Butz S, Okamoto M, Südhof TC. A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell.* 1998 Sep 18;94(6):773-82. doi:10.1016/s0092-8674(00)81736-5.
2. Zhang K, Wang T, Liu X, Yuan Q, Xiao T, Yuan X, Zhang Y, Yuan L, Wang Y. CASK, APBA1, and STXBP1 collaborate during insulin secretion. *Mol Cell Endocrinol.* 2021 Jan 15;520:111076. doi:10.1016/j.mce.2020.111076.
3. Mondal T, Loffredo CA, Simhadri J, Nunlee-Bland G, Korba B, Johnson J, Cotin S, Moses G, Quartey R, Howell CD, Noreen Z, Arif M, Ghosh S. Insights on the pathogenesis of type 2 diabetes as revealed by signature genomic classifiers in an African American population in the Washington, DC area. Diabetes Metab Res Rev. 2023; 39(1):e3589. doi:10.1002/dmrr.3589.
4. van der Klaauw AA, Croizier S, Mendes de Oliveira E, Stadler LKJ, Park S, Kong Y, Banton MC, Tandon P, Hendricks AE, Keogh JM, Riley SE, Papadia S, Henning E, Bounds R, Bochukova EG, Mistry V, O'Rahilly S, Simerly RB, Minchin JEN, Barroso I, Jones EY, Bouret SG, Farooqi IS. Human Semaphorin 3 Variants Link Melanocortin Circuit Development and Energy Balance. *Cell.* 2019 Feb 7;176(4):729-742.e18. doi:10.1016/j.cell.2018.12.009.
5. Zhu N, LeDuc CA, Fennoy I, Laferrère B, Doege CA, Shen Y, Chung WK, Leibel RL. Rare predicted loss of function alleles in Bassoon (BSN) are associated with obesity. *npj Genomic Medicine* 2023, 8:33. doi:10.1038/s41525-023-00376-7.

## Brain Development and Brain Function

# 11.11. Neurodevelopmental programming of adiposity: contributions to obesity risk

Skowronski AA, Leibel RL, LeDuc CA

Division of Molecular Genetics, Department of Pediatrics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY 10032, USA Naomi Berrie Diabetes Center, Columbia University Irving Medical Center, New York, NY 10032, USA

*Endocr Rev.* 2024 Mar 4;45(2):253-280.

doi:10.1210/endrev/bnad031.

https://pubmed.ncbi.nlm.nih.gov/37971140/

Brief summary: This review combined findings from human and rodent studies on the maternal factors that influence the neurodevelopmental programming of long-term adiposity. It highlights the roles of maternal nutritional status, metabolic hormones and epigenetic changes during gestation and the postnatal period on the programming of body weight in the offspring, and their impact on the development of hypothalamic, brain stem, and hedonic circuits within the central nervous system.

This review raises some critical considerations for obesity and obesity-related disease prevention. Maternal obesity or overnutrition during the prenatal and postnatal periods are major determinants in programming body weight in the offspring. Effects of maternal obesity can accumulate through epigenetic marks over successive generations to shift the population distribution toward increased adult body weight (1). Optimization of maternal body mass/composition and nutrition during the third trimester and neonatal nutrition are important goals to decrease the lifetime obesity risk in offspring.

In utero and early postnatal insulin and leptin exposure is another important contributor in adiposity programming. Exposure to elevated levels of insulin is associated with increased body weight gain, glucose impairment and altered hypothalamic circuits in the offspring (2, 3). Both reduced and augmented leptin surges during the immediate postnatal period are associated with increased adult body weight (4). Fetal hyperinsulinemia due to maternal hyperglycemia is associated with increased fat accrual and higher fetal circulating leptin concentration. It can be concluded that an optimal concentration of these hormones is desirable during early life.

Current interventions in obese women have only a mild or no effect on offspring outcomes regarding birth weight and fat accumulation (5, 6). Understanding the complex neuro-molecular pathways for the developmental programming of adiposity may help to design preventive strategies as well as therapeutic approaches to prevent the transgenerational transmission of obesity. Reducing body weight and improving metabolic health in women prior to conception could have the most profound impact, not only on maternal and offspring outcomes, but also on the health of future generations.

References

1. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond).* 2008;32(9):1373-9.

2. Plagemann A, Heidrich I, Götz F, Rohde W, Dörner G. Lifelong enhanced diabetes susceptibility and obesity after temporary intrahypothalamic hyperinsulinism during brain organization. *Exp Clin Endocrinol.* 1992;99(2):91-5.
3. Carmody JS, Wan P, Accili D, Zeltser LM, Leibel RL. Respective contributions of maternal insulin resistance and diet to metabolic and hypothalamic phenotypes of progeny. *Obesity (Silver Spring).* 2011;19(3):492-9.
4. Attig L, Solomon G, Ferezou J, Abdennebi-Najar L, Taouis M, Gertler A, et al. Early postnatal leptin blockage leads to a long-term leptin resistance and susceptibility to diet-induced obesity in rats. *Int J Obes (Lond).* 2008;32(7):1153-60.
5. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. *Bmj.* 2014;348:g1285.
6. Grivell RM, Yelland LN, Deussen A, Crowther CA, Dodd JM. Antenatal dietary and lifestyle advice for women who are overweight or obese and the effect on fetal growth and adiposity: the LIMIT randomised trial. *Bjog.* 2016;123(2):233-43.

## 11.12. Age-related ciliopathy: obesogenic shortening of melanocortin-4 receptor-bearing neuronal primary cilia

Oya M, Miyasaka Y, Nakamura Y, Tanaka M, Suganami T, Mashimo T, Nakamura K
Department of Integrative Physiology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japankazu@med.nagoya-u.ac.jp.
*Cell Metab.* 2024 May;36(5): 1044-1058.e10.
doi:10.1016/j.cmet.2024.02.010.
https://pubmed.ncbi.nlm.nih.gov/38452767/

Brief summary: This study describes how the primary cilia of hypothalamic neurons bearing melanocortin-4 receptors (MC4R) progressively shorten with age, leading to reduced sensitivity to satiety signals, increased appetite, and obesity. This age-related ciliary shortening is exacerbated by chronic leptin-melanocortin signalling, but can be mitigated by dietary restriction or knockdown of ciliogenesis-associated kinase 1 (CILK1). These findings suggest potential therapeutic approaches to combat obesity and metabolic syndrome in aging populations.

Primary cilia play crucial roles in various signaling pathways, including those involved in metabolic regulation[1]. The identification of chronic leptin-melanocortin signaling as a promoter of ciliary shortening highlights a feedback mechanism where prolonged activation of this pathway leads to reduced effectiveness, contributing to obesity. This insight is particularly relevant as it connects hyperleptinemia and leptin resistance, commonly observed in obese individuals, to structural changes in hypothalamic neurons. The reversal or inhibition of ciliary shortening through dietary restriction or CILK1 knockdown indicates that interventions at the molecular level can restore ciliary function and improve metabolic outcomes. This opens avenues for drug development to prevent ciliary shortening, and thereby enhance sensitivity of hypothalamic neurons to satiety signals and promoting energy expenditure. Investigating whether similar mechanisms occur in other types of neurons and tissues could also provide a more comprehensive understanding of aging and obesity.

Reference

1. DeMars KM, Ross MR, Starr A, McIntyre JC. Neuronal primary cilia integrate peripheral signals with metabolic drives. *Front Physiol* 2023; 14: 1-14.

## 11.13. The insulin resistant brain: impact on whole-body metabolism and body fat distribution

Heni M
Division of Endocrinology and Diabetology, Department of Internal Medicine 1, University Hospital Ulm, Ulm, Germany; Department for Diagnostic Laboratory Medicine, Institute for Clinical Chemistry and Pathobiochemistry, University Hospital of Tübingen, Tübingen, Germany.
martin.heni@uniklinik-ulm.de
*Diabetologia*. 2024 Jul;67(7):1181-1191.
doi:10.1007/s00125-024-06104-9.
https://pubmed.ncbi.nlm.nih.gov/38363340/

Brief summary: This review describes progress of our understanding of insulin action in the brain, as well as the consequences of brain insulin resistance. Insulin resistance of the brain results in impaired modulation of

peripheral metabolism, the maintenance of obesity and an unfavorable body fat distribution. Current evidence suggest that brain insulin resistance is a treatable condition, thereby improving systemic metabolism and brain functions including cognition.

The human brain plays a central role in the regulation of glucose and lipid metabolism, body weight and fat distribution. When insulin is administered transnasally, it reaches the brain and has dose-dependent effects there, particularly in the hypothalamus. This influences lipid metabolism, such as fat distribution, and is important for homeostasis, food intake and cognition.

The human brain can suppress endogenous glucose production and stimulate glucose uptake in peripheral tissues such as muscles and adipocytes. It also influences insulin secretion by the pancreas. After food intake, insulin enters the brain, which sends signals to the periphery to suppress glucose production in the liver and promote insulin secretion. Disruptions in these signaling pathways can lead to a high risk of complications and increased mortality. The question how these signals are transmitted from the brain to peripheral tissues is key to understanding metabolic regulation.

## Adipocyte Dysfunction and Obesity Related Comorbidities

### 11.14. Obesity causes mitochondrial fragmentation and dysfunction in white adipocytes due to RalA activation

Xia W, Veeragandham P, Cao Y, Yayun Xu Y, Rhyne TE, Qian J, Chao-Wei Hung, CHZhao P, Jones Y, Hui Gao H, Liddle C, Yu RT, Downes M, Evans RM, Rydén M, Wabitsch M, Wang Z, Hakozaki H, Schöneberg J, Reilly SM, Huang J, Saltiel AR
Division of Endocrinology and Metabolism, Department of Medicine, University of California San Diego, San Diego.
asaltiel@health.ucsd.edu
*Nat Metab*. 2024; 6(2): 273-289.
doi:10.1038/s42255-024-00978-.
https://pubmed.ncbi.nlm.nih.gov/38286821/

Brief summary: This study shows that obesity induces mitochondrial fragmentation and reduces oxidative capacity in white adipocytes through the activation of the small GTPase RalA. Targeted deletion of RalA in these cells prevents mitochondrial fragmentation, improves energy expenditure, and protects against obesity-induced metabolic dysfunctions, highlighting the critical role of RalA in obesity-related mitochondrial and metabolic abnormalities.

Previous research established that mitochondrial dysfunction is a hallmark of obesity, contributing to insulin resistance and other metabolic disorders[1]. This study extends that knowledge by elucidating a specific molecular mechanism involving RalA activation, which leads to mitochondrial fragmentation in white adipocytes. RalA, a member of the Ras superfamily of GTPases, is involved in various cellular processes including proliferation and cell survival in cancer context[2]. Its specific role in adipocyte metabolism and mitochondrial dynamics was unclear. Here, targeted deletion of RalA in white adipocytes prevented mitochondrial fragmentation, enhanced oxidative capacity, and increased energy expenditure via Drp1 phosphorylation. Further, RalA knockout protected mice on high-fat diet from development of insulin resistance. These findings have broader implications for metabolic research beyond obesity. The identification of a new regulatory axis involving RalA and Drp1 in mitochondrial dynamics may be relevant to other metabolic tissues and conditions. This could lead to a deeper understanding of mitochondrial biology and its role in other metabolic diseases.

References

1. Chen W, Zhao H, Li Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduct. *Target. Ther.* 2023; 8. doi:10.1038/s41392-023-01547-9.
2. Yan C, Theodorescu D. RAL GTpases: Biology and potential as therapeutic targets in cancer. *Pharmacol Rev* 2018; 70: 1–11.

### 11.15. A spatiotemporal proteomic map of human adipogenesis

Klingelhuber F, Frendo-Cumbo S, Omar-Hmeadi M, Massier L, Kakimoto P, Taylor AJ, Couchet M, Ribicic S, Wabitsch M, Messias AC, Iuso A, Müller TD, Rydén M, Mejhert N, Krahmer N

Institute for Diabetes and Obesity, Helmholtz Zentrum München, Neuherberg, Germany.
Natalie.krahmer@helmholtz-munich.de
*Nat Metab* 2024 6(5):861-79.
doi:10.1038/s42255-024-01025-8.
https://pubmed.ncbi.nlm.nih.gov/38565923

Brief summary: This study created a temporally- and spatially-resolved proteomic atlas of human adipogenesis. It highlights cell restructuring and spatial reorganization of metabolic pathways to optimize cells for lipid accumulation and identifies C19orf12 as a differentiation-induced protein that regulates lipid storage in adipocytes.

This study provides a comprehensive and detailed proteomic map that captures the dynamic changes in protein abundance and localization during human adipogenesis. This is particularly important because previous research primarily focused on transcriptomic analyses, which do not fully capture post-transcriptional regulation and protein dynamics. This study identifies several key proteins and metabolic pathways involved in adipogenesis. For example, C19orf12, a protein previously associated with neuronal disorders[1], is shown to regulate lipid storage and mitochondrial function in adipocytes. This finding adds a new dimension to our understanding of how lipid storage capacity is regulated at the protein level, which is crucial for addressing metabolic disorders like obesity and diabetes. The spatiotemporal aspect of the proteomic map is a novel angle, which provides insights into the longitudinal regulation of metabolic pathways in adipose tissue. This level of detail helps elucidate how different cellular compartments coordinate during adipocyte differentiation, and offers potential targets for therapeutic intervention in metabolic diseases.

This proteomic atlas is a valuable resource for future studies aiming to explore the functional roles of identified proteins in adipogenesis and metabolic regulation. It may open avenues for investigating how genetic variations and environmental factors influence adipocyte function at the proteomic level.

Reference

1. Gagliardi M, Annesi G, Lesca G, Broussolle E, Iannello G, Vaiti V et al. C19orf12 gene mutations in patients with neurodegeneration with brain iron accumulation. *Park Relat Disord* 2015; 21: 813-816.

## 11.16. The role of adipogenic capacity and dysfunctional subcutaneous adipose tissue in the inheritance of type 2 diabetes mellitus: cross-sectional study

Šiklová M, Šrámková V, Koc M, Krauzová E, Čížková T, Ondrůjová B, Wilhelm M, Varaliová Z, Kuda O, Neubert J, Lambert L, Elkalaf M, Gojda J, Rossmeislová L
Department of Pathophysiology, Third Faculty of Medicine, Charles University, Ruska 87, 100 00 Prague 10, Czech Republic.
michaela.siklova@lf3.cuni.cz
*Obesity (Silver Spring)*. 2024;32:547–559.
https://doi.org/10.1002/oby.23969, https://pubmed.ncbi.nlm.nih.gov/38221680/

Brief summary: This cross-sectional study in n = 19 first-degree relatives of type 2 diabetes mellitus (T2DM) patients and n = 19 control individuals without obesity found that while the intrinsic adipogenic potential of subcutaneous adipose tissue (SAT) is unaffected by a family history of T2DM, alterations in lysyl oxidase (LOX) mRNA expression and polyunsaturated fatty acids in triglycerides are linked to increased T2DM risk, independent of obesity. These findings suggest that SAT dysfunction, rather than its expansion capacity, may predispose individuals to T2DM.

This study challenges the prevailing notion that reduced adipogenic capacity is the primary driver of subcutaneous adipose tissue (SAT) hypertrophy in first-degree relatives of patients with type 2 diabetes mellitus (T2DM) (1, 2). Contrary to previous findings, this study indicates that the intrinsic adipogenic potential of SAT remains intact in these predisposed individuals before fat mass accumulation. Instead, the study identifies novel markers—specifically increased polyunsaturated fatty acids in triglycerides (PUFA-TAGs) and lysyl oxidase (LOX) mRNA expression—as critical factors linked to SAT dysfunction. These markers are associated with increased visceral fat, insulin resistance, and cellular stress, suggesting that they may contribute to the development of T2DM independent of traditional obesity pathways.

These findings underscore the need to explore SAT dysfunction beyond adipogenic capacity in the pathophysiology of T2DM. However, the study has some limitations, including a relatively small sample

size, particularly when examining complex metabolic pathways and genetic predispositions. Additionally, the study focuses on male participants without obesity, which may limit its generalizability to other populations, including women and those with obesity.

Future research should explore these findings in larger, more diverse cohorts to validate the biomarkers identified and investigate their potential in clinical settings. Furthermore, longitudinal studies are needed to assess how these early markers of SAT dysfunction and visceral fat accumulation translate into the development of T2DM over time. The interplay between genetic predisposition, environmental factors, and adipose tissue dysfunction remains a critical area for future exploration to fully understand and mitigate the risk of T2DM.

References

1. Arner P, Arner E, Hammarstedt A, Smith U. Genetic predisposition for type 2 diabetes, but not for overweight/obesity, is associated with a restricted adipogenesis. *PLoS One.* 2011;6:e18284.
2. Henninger AM, Eliasson B, Jenndahl LE, Hammarstedt A. Adipocyte hypertrophy, inflammation and fibrosis characterize subcutaneous adipose tissue of healthy, non-obese subjects predisposed to type 2 diabetes. *PLoS One.* 2014;9:e105262.

## 11.17. At any level of adiposity, relatively elevated leptin concentrations are associated with decreased insulin sensitivity

Chiriacò M, Nesti L, Flyvbjerg A, Golay A, Nazare J-A, Anderwald C-H, Mitrakou A, Bizzotto R, Mari A, Natali A
Metabolism, Nutrition, and Atherosclerosis Laboratory, Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy. dxf1@cdc.gov

*J Clin Endocrinol Metab*. 2024 Jan 18;109(2):461-470.
doi:10.1210/clinem/dgad505.
https://pubmed.ncbi.nlm.nih.gov/37650623/

Brief summary: This study compared 2 groups of adults defined by their leptin concentrations relative to fat mass. Adults with 'relatively high leptin' (RHL, n = 646) or 'relatively low leptin' (RLL, n = 644) concentrations were compared in insulin concentration, insulin secretion, and insulin sensitivity, at both the whole-body level and within adipose tissue. Individuals with RHL showed a distinct metabolic phenotype, characterized by insulin resistance in both whole-body glucose metabolism and adipose tissue lipolysis, regardless of their degree of adiposity.

There is a high variability in leptin concentrations between individuals with the same BMI or fat mass. Whether individuals with RHL or RLL differ in terms of their metabolic phenotype and response to interventions is of clinical interest. As there are no reference values for leptin concentrations that cover the entire BMI spectrum in adults, the authors used a sex-specific leptin-fat mass regression within the studied cohort to categorise adults as having RLL or RHL. In another study, Akinci et al. grouped adults with leptin concentrations $<$25th percentile of NHANES III population based on sex and BMI as RLL and adults with leptin concentrations $>$75th percentile as RHL (1). Potential therapeutic consequences of RLL were suggested by Akinci et al., who showed that exogenous leptin therapy in adults with RLL reduced hepatic steatosis. Together, these findings support the idea of a metabolic phenotype characterized by relative leptin concentrations. The development of reference values for leptin concentrations according to sex, age, BMI or fat mass would allow more insights into these metabolic subtypes.

Reference

1. Akinci, B.; Subauste, A.; Ajluni, N.; Esfandiari, N.H.; Meral, R.; Neidert, A.H.; Eraslan, A.; Hench, R.; Rus,D.; McKenna, B.; Hussain, H.K.; Chenevert, T.L.; Tayeh, M.K.; Rupani, a.R.; Innis, J.W.; Mantzoros, C.S.; Conjeevaram , H.S.; Burant, C.L.; Oral E.A. Metreleptin therapy for nonalcoholic steatohepatitis: Open-label therapy interventions in two different clinical settings? *Med* 2021, 9, 2(7):814-835. doi:10.1016/j.medj.2021.04.001.

# 12. Type 2 Diabetes, Metabolic Syndrome and Lipids

Orit Pinhas-Hamiel, MD.
Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children s Hospital, Sheba Medical Center, Ramat-Gan, and Juvenile Diabetes Center, Maccabi Health Care Services, Tel-Aviv University, Sackler School of Medicine, Israel

## Concerns in T2D

### 12.1. Major adverse events in youth-onset type 1 and type 2 diabetes: the SEARCH and TODAY studies

Mottl AK, Tryggestad JB, Isom S, Gubitosi-Klug RA, Henkin L, White NH, D'Agostino R Jr, Hughan KS, Dolan LM, Drews KL SEARCH for Diabetes in Youth Study Group; TODAY Study Group.
*Diabetes Res Clin Pract*. 2024 Apr;210:111606.
doi:10.1016/j.diabres.2024.111606.

Brief summary: This longitudinal observational study combined data from the SEARCH (T1D: N = 564; T2D: N = 149) and TODAY studies (T2D: N = 495). Complications were higher in those with childhood onset T2D compared to T1D, with T2D showing 2.5 to 4 times higher rates of microvascular, macrovascular events despite similar diabetes duration. Risk factors for complications differed between T2D and T1D, with mean arterial pressure being a common predictor for both.

Comment: The SEARCH for Diabetes in Youth (SEARCH) study is an observational study initiated in 2000 at five sites across the U.S., designed to estimate the prevalence, incidence, and complications of both T1D and T2D in youth. The Treatment Options for T2D in Adolescents and Youth (TODAY(study is an interventional study focused specifically on adolescents with T2D aiming to compare the effectiveness of different treatment options for managing the disease in this age group. Here, the researchers combined data from these 2 studies to assess rates of micro- and macrovascular complications in 564 youth-onset T1D and 644 youth-onset T2D, creating the largest sample of its kind in children. It also assessed rates of medical events related to inflammation and insulin resistance, including venous thrombosis, pancreatitis, gallbladder disease, and cirrhosis.

Mean age at assessment ranged between 21 and 26 years, and mean disease duration was 11-13 years. All complications were higher in youth with T2D than T1D. Individuals with youth-onset T2D developed micro- or macrovascular disease earlier in their disease course, starting about 8 years after diagnosis. In youth-onset T2D, blood pressure was the sole predictor of both micro- and macrovascular disease. In another recent study, of 196 participants with youth-onset T2D and hypertension or nephropathy, 157 (80.1%) had low adherence.[1] Of participants with low adherence, 106 (67.52%) were not using any blood pressure-lowering medication.

These data emphasize the need for a proactive and assertive approach to prevent the complications associated with T2D in adolescents.

Reference

1. Weinstock RS, Trief PM, Burke BK, Wen H, Liu X, Kalichman S, Anderson BJ, Bulger JD. Antihypertensive and Lipid-Lowering Medication Adherence in Young Adults With Youth-Onset Type 2 Diabetes. *JAMA Netw Open.* 2023 Oct 2;6(10):e2336964. doi:10.1001/jamanetworkopen.2023.36964.

### 12.2. Trajectories of eGFR and risk of albuminuria in youth with type 2 diabetes: results from the TODAY cohort study

El Ghormli L, Wen H, Uschner D, Haymond MW, Hughan KS, Kutney K, Laffel L, Tollefsen SE, Escaname EN, Lynch J, Bjornstad P, TODAY Study Group

*Pediatr Nephrol*. 2023 Dec;38(12):4137-4144.
PubMed: 37434027.

Brief summary: The authors performed exploratory analyses to identify distinct eGFR trajectories associated with risk of albuminuria in 377 adolescents with T2D. The findings underscore the importance of annual GFR assessments in young people with T2D.

Comment: Diabetic kidney disease (DKD) is the primary microvascular complication in adolescents with T2D, and is a leading cause of morbidity and mortality. Albuminuria is the earliest clinical indicator of DKD. Data from adults with T2D indicate that the natural history of DKD often begins with hyperfiltration—an elevated glomerular filtration rate (GFR) exceeding 120 mL/min/1.73 $m^2$—due to obesity and impaired glucose tolerance. This hyperfiltration stage predicts kidney function decline even before other clinical signs emerge. The second stage in the progression of kidney dysfunction, still asymptomatic, involves a mild reduction in GFR (60-89 mL/min/1.73 $m^2$). During this phase, structural changes in the kidney are common but often reversible, highlighting a crucial window for risk factor modification.

The understanding of early DKD progression in adolescents with youth-onset T2D is still limited. This study delineated distinct hyperfiltration trajectories and their association with albuminuria in youth-onset T2D, with a follow-up duration of at least 10 years. GFR trajectories and eGFR peaks were analyzed to more accurately capture glomerular hyperfiltration patterns. It included 377 adolescents (63%) with T2D. At baseline, the median age was 14 years, the median diabetes duration was 6 months, the mean BMI was 34.9 kg/$m^2$, the mean HbA1c was 6.0%, and the mean eGFR was 120 mL/min/1.73 $m^2$.

Five distinct eGFR trajectories were identified. The first 3 groups showed relatively stable eGFR levels, differing mainly in their baseline eGFR values of 108, 118, and 131 mL/min/1.73 $m^2$, respectively. The fourth group began with a baseline eGFR of 141 mL/min/1.73 $m^2$, which increased to 176 mL/min/1.73 $m^2$ by year 10. In contrast, the fifth group showed a progressive decline in eGFR, dropping below 60 mL/min/1.73 $m^2$ by the end of the study. There were no major differences in stratification of eGFR when data was restricted to those with albuminuria (n = 150).

These data suggest that in young people with T2D, a continuous and increasing filtration rate might be a warning sign of DKD. However, we need more time to see if this increased filtration will eventually lead to a decline in kidney function. Notably, the group with progressively increasing eGFR, which showed the highest peak eGFR and albuminuria levels, tended to be female. This contrasts with observations in adult-onset T2D, where males typically have higher risk of albuminuric DKD. Patients who showed the greatest peak hyperfiltration also showed the highest degree of albuminuria both at baseline and after 10 years of follow-up. The median time to peak eGFR in this group was 8 years.

## 12.3. Age at type 2 diabetes diagnosis and cause-specific mortality: observational study of primary care patients in England

Barker MM, Davies MJ, Sargeant JA, Chan JCN, Gregg EW, Shabnam S, Khunti K, Zaccardi F
*Diabetes Care*. 2023 Nov 1;46(11):1965-1972.
doi:10.2337/dc23-0834.

Brief summary: This population-based cohort study estimated all-cause and cause-specific mortality in 108,061 individuals with newly diagnosed T2D, (16–50 years of age) compared to 829,946 individuals without T2D. Higher relative risks of mortality in younger individuals with vs. without T2D ranged from 4 to 11, depending on the cause of death.

Comment: A decade ago, a Markov-like computer model simulated the life course of a hypothetical cohort of U.S. adolescents and young adults aged 15–24 years newly diagnosed with T2D. The model predicted that while a 20-year-old without diabetes has an average remaining life expectancy of 58 years, those with T2D can expect only 43 years - a loss of ∼15 years. The model also suggested that these individuals might face severe, chronic complications emerging by their 40s.

Now, this large, multiethnic, nationally representative cohort study confirms those grim predictions. Across all causes of death, mortality risk was consistently higher in individuals with T2D. The relative risk of all-cause mortality was particularly alarming in those aged 16 to 27, where it was more than 4-times higher, and gradually declined to 1.5 in the 48- to 50-year age group. Cardiorenal mortality, risk was even more severe, soaring over 10-fold in the youngest group and tapering to 2-fold in those nearing 50. Cancer-specific mortality also peaked among the youngest individuals, with the relative risk reaching 3.7 in those aged 16 to 27. Individuals with T2D had higher BMI, systolic blood pressure, and LDL level, reported more active smoking and were more likely to be in the most deprived socioeconomic quintile.

Although the absolute risk of events in individuals diagnosed with T2D at a young age was low, these findings underscore the need for strategies focused on screening for T2D at younger ages and tailoring therapeutic approaches to prevent diabetes-related complications and mortality in this population.

Reference

1. Rhodes ET, Prosser LA, Hoerger TJ, Lieu T, Ludwig DS, Laffel LM. Estimated morbidity and mortality in adolescents and young adults diagnosed with Type 2 diabetes mellitus. *Diabet Med.* 2012 Apr;29(4):453-63. doi:10.1111/j.1464-5491.2011.03542.x. PMID: 22150528.

## Improving T2D Outcomes

### 12.4. Continuous glucose monitoring versus standard of care in adolescents with type 2 diabetes: a pilot randomized cross-over trial

Chang N, Barber ROB, Llovido Alula J, Durazo-Arvizu R, Chao LC

*J Diabetes Sci Technol*. 2023 Sep;17(5):1419-1420.
doi:10.1177/19322968231178284.

Brief summary: This pilot study assessed the feasibility and impact of continuous glucose monitoring (CGM) in 9 adolescents with T2D. CGM use lowered HbA1c by up to 3.8% in participants who consistently used the device.

Comment: Large cohort studies investigating complications in children and adolescents with T2D have clearly shown a higher incidence of complications compared to both adults with T2D and children with type 1 diabetes (T1D). These complications often emerge at an early age and are strongly associated with poor glycemic control.

Among children and adolescents with T1D, the use of continuous glucose monitors (CGMs) alone improves outcomes and is now considered standard of care. This pilot study assessed the feasibility of CGM use and its effect on glycemic outcomes in youth with T2D. Nine adolescents, mean age $17.4 \pm 2.0$ years, with a T2D duration of $4.0 \pm 2.8$ years, and HbA1c $11.5 \pm 2.5\%$. were randomized to 3 months of Dexcom G6 CGM or blood glucose monitoring (BGM), followed by 3 months washout period and then crossed over to the other arm for 3 months.

CGM use lowered HbA1c by 2.8% in the CGM group, and by 3.8% for those who wore the CGM $> 85\%$ of the time. Despite being a preliminary investigation with a very small sample, its findings are notable. It appears that the adolescent community with T2D, despite being at high-risk for early complications, is not receiving the level of care it requires. In a study assessing whether clinicians are sufficiently aggressive in treating diabetes-related dyslipidemia in youth, only 5% of those with dyslipidemia were prescribed lipid-lowering medications.[2] Similarly, in the TODAY study cohort, only half of the youth with hypertension were on blood pressure-lowering medication. While it may be easy to attribute this to patient behavior, it is also possible that because, unlike T1D where there is an immediate risk of ketoacidosis, and due to outdated perceptions that complications in T2D appear later in life, healthcare teams may not be adopting an aggressive enough approach.

A larger, more comprehensive study is needed to assess the impact of recent technologies in adolescents with T2D.

References

1. Schoelwer, M.J., DeBoer, M.D. & Breton, M.D. Use of diabetes technology in children. *Diabetologia* (2024). https://doi.org/10.1007/s00125-024-06218-0.
2. Jackson S, Creo A, Kumar S. Are Clinicians Aggressive Enough in Treating Diabetes-Related Hyperlipidemia in Youth? *Curr Atheroscler Rep.* 2022 Jun;24(6):471-481. doi:10.1007/s11883-022-01020-y.

## 12.5. The efficacy and safety of GLP-1 receptor agonists in youth with type 2 diabetes: a meta-analysis

Yugar LBT, Sedenho-Prado LG, da Silva Ferreira IMC, Silva CAM, Sposito AC, Cercato C

*Diabetol Metab Syndr.* 2024 Apr 24;16(1):92.
doi:10.1186/s13098-024-01337-5.

Brief summary: This meta-analysis including 415 children and adolescents with T2D showed that GLP-1 receptor agonists reduced HbA1c by 1%, fasting blood glucose, and body weight by 1.6 kg, with no notable impact on blood pressure. An increased incidence of mainly gastrointestinal side effects was observed.

Comment: Over the past decade, glucagon-like peptide 1 (GLP-1) receptor agonists have revolutionized the management of T2DM in adults.[1] These drugs not only improve glycemic control, but also promote weight loss, reduce the risk of major cardiovascular events and provide kidney protection. In contrast, trials in adolescents with T2D have demonstrated an improvement in HbA1c levels compared to untreated groups, but have not shown significant changes in BMI or blood pressure. This meta-analysis aimed to address the gaps by increasing sample size and statistical power, thereby providing more precise estimates of the effects of these interventions.

Five randomized clinical trials were included, encompassing a total of 415 patients, with follow-up ranging 5 to 26 weeks. Mean age ranged from 14.5 to 15.8 years, and two-thirds were females. Baseline mean HbA1c ranged 7.9% to 8.3%, mean body weight 89 kg to 101 kg, and mean body mass index (BMI) 34 to 37 kg/m$^2$.

In the meta-analysis, GLP-1 RA was found to lower HbA1c levels by -1%, and fasting glucose levels by 34 mg/dL. Only 3/5 studies reported data on body weight. While GLP-1 RA led to mean weight reduction of -1.6 kg compared to placebo, this change is marginal given the degree of obesity in this population. GLP-1 RA had no impact on blood pressure in adolescents. The limited number of trials for each GLP-1 RA makes it unclear if any medication is superior to another.

The limited weight loss among adolescents on GLP-1 receptor agonists is not fully understood. It is possible that the daily injections are burdensome, that socioeconomic circumstances are challenging, and the side effects significant. Adolescents in the intervention group had twice the odds of adverse effects, such as nausea, vomiting, and diarrhea. It is hoped that newer treatments - such as other GLP-1 receptor agonists, dual agonists triple combination therapies, and potent oral agonists — will offer better outcomes than those achieved with currently available agents

Reference

1. Yao H, Zhang A, Li D, Wu Y, Wang CZ, Wan JY, Yuan CS. Comparative effectiveness of GLP-1 receptor agonists on glycaemic control, body weight, and lipid profile for type 2 diabetes: systematic review and network meta-analysis. *BMJ.* 2024 Jan 29;384:e076410. doi:10.1136/bmj-2023-076410.

## 12.6. Tirzepatide once weekly for the treatment of obesity in people with type 2 diabetes (SURMOUNT-2): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial

Garvey WT, Frias JP, Jastreboff AM, le Roux CW, Sattar N, Aizenberg D, Mao H, Zhang S, Ahmad NN, Bunck MC, Benabbad I, Zhang XM; SURMOUNT-2 investigators.

*Lancet*. 2023 Aug 19;402(10402):613-626.
doi:10.1016/S0140-6736(23)01200-X.

Brief summary: This double-blind, placebo-controlled international trial, randomized overweight and obese adults with T2D to either once-weekly subcutaneous Tirzepatide (10 mg or 15 mg) or placebo for 72 weeks. Tirzepatide led to weight reductions of -9.6% with the 10 mg dose and -11.6% with the 15 mg dose, compared to placebo. From a mean baseline 8.0%, HbA1c reduced by –2·1% with both the 10 mg, and 15 mg dose of triszepatied compared to 0·5% reduction on placebo.

Comment: Tirzepatide is the first of a new class of drugs able to selectively bind and activate the receptors for both the intestinal hormones, glucagon like peptide 1 (GLP1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 is secreted by L cells located in the distal ileum and colon in response to nutrient ingestion. Activation of GLP-1-associated signalling pathways leads to increased insulin secretion and reduced glucagon production, enhancing glucose utilization in insulin-dependent tissues such as muscle and adipose tissue. Additionally, GLP-1 slows gastric emptying, modulates calorie intake by increasing the sense of satiety, influences cardiovascular activity, regulates natriuresis at the renal level, and has neuroprotective effects.[1]

GIP is secreted by K-cells in the duodenum and upper jejunum following the oral ingestion of nutrients such as glucose, amino acids, and long-chain fatty acids. Its administration reduces β-cell apoptosis and enhances β-cell mass in animal models. GIP administration results in decreased food intake and has beneficial effects on bone mass.

Tirzepatide has a greater affinity for GIP receptors than for GLP-1 receptors. This dual agonist effect results in greater reductions in weight and glucose levels compared to a selective GLP-1 receptor agonist. It was approved for the treatment of T2D in adults in 2022 and for weight loss in 2023.

Since individuals with obesity and T2D often experience less weight reduction with anti-obesity medications than those without diabetes, the current study evaluated Tirzepatide in adults with obesity and T2D. Among those on 15 mg Tirzepatide, 51.8% achieved $\geq$15% weight reduction and 34.0% achieved $\geq$20% weight reduction, compared to 2.6% and 1.0% with placebo, respectively. The % of participants achieving an HbA1c level of $<$5.7% was 55% for the 10 mg dose and 50% for the 15 mg dose, compared to 2.8% with placebo. Additionally, there were decreases in waist circumference, systolic blood pressure, fasting triglycerides, and HDL-cholesterol.

We await the results of the SURPASS-PEDS study, which is evaluating Tirzepatide in children aged 10 to under 18 years with T2D inadequately controlled with metformin, basal insulin, or both, and the SURMOUNT-ADOLESCENTS-2 study, which is assessing Tirzepatide's impact on body weight and cardiovascular risk factors in adolescents with obesity and weight-related comorbidities.

Reference

1. Ciardullo S, Morieri ML, Daniele G, Fiorentino TV, Mezza T, Tricò D, Consoli A, Del Prato S, Giorgino F, Piro S, Solini A, Avogaro A. GLP1-GIP receptor co-agonists: a promising evolution in the treatment of type 2 diabetes. Acta Diabetol. 2024 Aug;61(8):941-950. doi:10.1007/s00592-024-02300-6.

## Genetics of T2D in Children and Adolescents

### 12.7. Genetic architecture and biology of youth-onset type 2 diabetes

Kwak SH, Srinivasan S, Chen L, Todd J, Mercader JM, Jensen ET, Divers J, Mottl AK, Pihoker C, Gandica RG, Laffel LM, Isganaitis E, Haymond MW, Levitsky LL, Pollin TI, Florez JC, Flannick J
Progress in Diabetes Genetics in Youth (ProDiGY) consortium.

*Nat Metab*. 2024 Feb;6(2):226-237.
doi:10.1038/s42255-023-00970-0.

Brief summary: Rare forms of diabetes ('monogenic diabetes') are caused by a single rare gene variant, while adult-onset T2D is influenced by thousands of common genetic variants. This study reveals that youth-onset T2D shares some genetic features with both monogenic and typical adult-onset T2D, marked by both common and rare genetic variants

Comment: A significant challenge is understanding why the course of T2D in children and teenagers is more aggressive compared to both adult-onset T2D and T1D in adolescents. A possible explanation may lie in the different genetic backgrounds and biological pathways between these conditions. These authors perform detailed genetic characterization of youth-onset T2D by analysing exome sequences and common variant associations in 3,005 individuals with youth-onset T2D and 9,777 adult controls matched for sex and ethnicity.

Firstly, in exome sequence data, 2.4% of those with youth-onset T2D had monogenic diabetes — arising from a single gene variant - including several individuals who carried variants in either *MC4R*, a gene strongly linked to monogenic obesity, or *HNF1A*, linked to monogenic diabetes. This suggests that individuals with youth-onset T2D may need screening for monogenic disease, which will inform appropriate medical treatment.

Next, exome sequences were compared between youth-onset and adult-onset T2D. The the combination of common and rare genetic variants exerts a greater influence on youth-onset T2D risk than adult-onset T2D. Individuals with youth-onset T2D were 3-times more likely to harbor common variants, compared with adults with T2D, and 5-times more likely to harbor rare gene variants. These data indicate that genetics plays a greater role in causing youth-onset T2D than it does in the adult-onset form. Genetic risk factors for youth-onset T2D did not overlap with those for lipodystrophies or T1D.

Finally, the specific mix of gene variants correlated with clinical presentation of youth-onset T2D. For example, those with more common variants showed more features of adult-onset T2D, such as high insulin levels, indicative of insulin resistance.

## Pregnancy and Youth Onset T2D

### 12.8. Impact of youth onset type 2 diabetes during pregnancy on microvascular and cardiac outcomes

Tryggestad JB, Drews KL, Mele L, Arslanian S, Chernausek SD, Escaname EN, Geffner M, Isganaitis E, Sprague J, Kelsey MM; TODAY Study Group.
*Diabetes Res Clin Pract*. 2023 Sep;203:110876.
doi:10.1016/j.diabres.2023.110876.

Brief summary: This observational study showed that pregnancy increases the risk of hyperfiltration in women with youth-onset T2D, but not other micro or macrovascular complications.

Comment: This study examined whether pregnancy exacerbates health outcomes in young women with T2D, particularly by testing for changes in the occurrence of microvascular and macrovascular complications. Previous findings showed that women in their fourth decade experience a notable increase in complications following pregnancy. Given the aggressive nature of youth-onset T2D, one might expect a similar impact of pregnancy on the incidence of complications. Neuropathy and nephropathy were assessed annually. Retinopathy, echocardiography, and arterial stiffness were assessed twice during the study.

Among women in the TODAY study, 116 reported a pregnancy lasting 20 weeks or more, with 67 experiencing a single pregnancy and 49 having multiple pregnancies. The average age at first pregnancy was 21.6 years, and the mean duration of diabetes was 8 years. Their data were compared to that of 291 nulliparous women with youth-onset T2D.

The encouraging news is that no difference was found in the rates of retinopathy, neuropathy, or macro- and microalbuminuria between women with and without a history of pregnancy. Additionally, pregnancy history did not affect echocardiographic measures or arterial stiffness when comparing pre- and post-pregnancy data.

However, women who had been pregnant were at higher risk of developing hyperfiltration, an early marker of nephropathy, (odds ratio: 2.76; CI: 1.38-5.49) compared to those who had not been pregnant. The median and interquartile range for the onset of nephropathy after pregnancy were: macroalbuminuria developed after 285 days (126–1255), microalbuminuria after 549 days (129–1607), and hyperfiltration after 375 days (302–935.5).

## 12.9. DNA methylation signatures of youth-onset type 2 diabetes and exposure to maternal diabetes

Salama OE, Hizon N, Del Vecchio M, Kolsun K, Fonseca MA, Lin DTS, Urtatiz O, MacIsaac JL, Kobor MS, Sellers EAC, Dolinsky VW, Dart AB, Jones MJ, Wicklow BA

*Clin Epigenetics*. 2024 May 13;16(1):65.

doi:10.1186/s13148-024-01675-1.

Brief summary: This cross-sectional analysis showed that DNA methylation (DNAm) patterns differ between youth-onset and adult-onset T2D. In utero exposure to maternal diabetes was linked to these distinct changes. These findings highlight unique molecular pathways disrupted by youth-onset T2D, and suggest potential intervention targets to prevent its health impacts.

Comment: Growing evidence suggests that T2D in children and adolescents is essentially different from that in adults. While adult-onset T2D is characterized by the gradual progression of insulin resistance and β-cell dysfunction, youth-onset T2D involves rapid deterioration in β-cell function and early onset of complications. An established risk factor for youth-onset T2D is exposure to diabetes during pregnancy. However, the mechanism linking in utero exposure to diabetes and risk of youth-onset T2D remains unclear. These authors hypothesized that DNA methylation may explain this difference.

DNA methylation is an epigenetic phenomenon, in which the C5 carbon of the cytosine residue attaches to a methyl group, predominantly at cytosine-phosphate-guanine (CpG) sites. This epigenetic alteration influences gene expression, and thereby, gene function. This study compared DNAm in youth-onset T2D (n = 218) and adolescents with normoglycemia (n = 77). They then investigated differences in youths exposed in utero to maternal diabetes (including gestational and pre-gestational diabetes) and youths who had a normoglycemic intrauterine environment.

This study identified 3,830 differentially methylated sites and 516 differentially methylated regions in peripheral blood with?> 1% difference in DNAm between youth with vs. without T2D, of which 36 sites and 17 DMRs displayed?more than 5% difference. Of these, 3 sites in *PFKFB3* were also associated with exposure to intrauterine maternal diabetes. These sites showed decreased DNAm in youth of mothers with T2D which was even lower in youth who had T2D themselves. PFKFB3 protein, predominantly expressed in endothelial cells, plays an important role in glycolysis. It is suggested that in utero exposure to maternal diabetes could affect the expression of *PFKFB3*, either in $\beta$-cells themselves or in other tissues, which in turn affects responsiveness to glucose.

Finally, comparing data with epigenome-wide association data on adult-onset T2D revealed that the majority of youth-onset T2D sites was not associated with adult-onset T2D, obesity, or youth obesity. This further supports the concept that youth-onset T2D is distinct from adult-onset T2D.

# Metabolic Syndrome

## 12.10. A proposed simplified definition of metabolic syndrome in children and adolescents: a global perspective

Zong X, Kelishadi R, Kim HS, Schwandt P, Matsha TE, Mill JG, Caserta CA, Medeiros CCM, Kollias A, Whincup PH, Pacifico L, López-Bermejo A, Zhao M, Zheng M, Xi B

*BMC Med*. 2024 May 7;22(1):190.

doi:10.1186/s12916-024-03406-y.

Brief summary: The authors propose a simplified definition of pediatric metabolic syndrome (MetS) for children aged 6-17 years. This uses static cut-offs, enabling easier and quicker assessment in clinical practice, and allowing comparison of MetS prevalence across different pediatric populations.

Comment: There is no consensus on the definition of pediatric metabolic syndrome (MetS). Existing pediatric MetS definitions involve age-, sex- or height-specific percentile cut-offs for central obesity and elevated blood pressure. This complicates the quick assessment of MetS risk and cardiometabolic risk factor clustering in clinical practice.

The proposed MetS definitions replace these percentile-based criteria with simplified static cut-offs (using absolute values) standardized across different ethnic populations. The new definition requires at least 3 of the following 5 components:

1. Central obesity: Waist-to-height ratio (WHtR) $\geq 0.50$ for youth from Europe and the USA, and $\geq 0.46$ for those from Asia, Africa, and South America.
2. High blood pressure: Systolic/diastolic blood pressure $\geq 130/80$ mm Hg for adolescents aged 13–17 years, and $\geq 120/80$ mm Hg for children aged 6-12 years
3. High triglycerides: $\geq 130$ mg/dl at age 10–17 years, and $\geq 100$ mg/dl at age 6–9 years
4. Low HDL-C: $< 40$ mg/dl
5. High fasting blood glucose: $\geq 100$ mg/dl

A validation study was done using individual data of 19,426 adolescents (50.8% males) aged 12–17 years, from Africa, Asia, Europe, North America and South Africa. Overall, MetS prevalence by the new simplified definition was 6.2%, roughly midway between that by the existing IDF (4.2%) and NCEP (7.7%) definitions.

As well as simplified definitions of MetS risk for population level monitoring, an 'action level' definition with more stringent cut-offs was proposed to guide clinical practice, to identify severely affected youths who require immediate intervention. Since measurements of waist circumference and blood pressure are not easily converted into age- and weight-adjusted percentiles, the use of absolute values seems highly practical.

## 12.11. Artemisinins ameliorate polycystic ovarian syndrome by mediating LONP1-CYP11A1 interaction

Liu Y, Jiang JJ, Du SY, Mu LS, Fan JJ, Hu JC, Ye Y, Ding M, Zhou WY, Yu QH, Xia YF, Xu HY, Shi YJ, Qian SW, Tang Y, Li W, Dang YJ, Dong X, Li XY, Xu CJ, Tang QQ
*Science*. 2024 Jun 14;384(6701):eadk5382.
doi:10.1126/science.adk5382.

Brief summary: Artemisinins, compounds known for their antimalarial properties, suppress ovarian androgen synthesis by promoting degradation of cytochrome P450 11A1 (CYP11A1). Hence, they are a promising new approach for preventing and treating PCOS.

Comment: Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism, chronic anovulation, and insulin resistance, is one of the most common reproductive endocrinopathies, affecting ~8-18% of reproductive-aged women.[1] Despite its high prevalence, the pathogenesis remain poorly understood and current treatments are nonspecific, primarily targeting symptom relief.

Artemisinin and its semisynthetic derivatives, originally discovered by Tu Youyou in 1972, are used in the treatment of malaria caused by Plasmodium falciparum. Tu Youyou, was awarded the 2015 Nobel Prize in Physiology or Medicine for this discovery. Artemisinin is extracted from the herb *Artemisia annua* (sweet wormwood), which gained notoriety due to its use in absinthe, a favorite French liqueur[2].

This study investigated the therapeutic potential of artemisinins in rodent models and human patients with PCOS, evaluating the effect of artemisinin derivatives on testosterone level, estrous cycle, and polycystic ovarian morphology. In rodent models, the artemisinin analogue artemether improved hyperandrogenemia, irregular estrous cycles, polycystic ovarian morphology, and low fertility. Artemisinins reduced hyperandrogenemia by inhibiting ovarian testosterone synthesis. The enzyme catalyzing the initial step of androgen synthesis is cytochrome P450 family 11 subfamily A member 1 (CYP11A1), which interacts with lon peptidase 1 (LONP1). Disruption of the interaction between LONP1 and CYP11A1 leads to the up regulation of CYP11A1, increasing androgen production and worsening PCOS. Artemisinins bind to LONP1, initiating interaction between LONP1 and CYP11A1, leading to degradation of CYP11A1, subsequently inhibiting ovarian androgen synthesis and curbing PCOS.

A pilot clinical trial was conducted to confirm the therapeutic effects of artemisinins in women with PCOS. Dihydroartemisinin reduced hyperandrogenemia, lowered anti-Müllerian hormone levels, improved polycystic ovarian morphology, and contributed to normalized menstrual cycles. A randomised controlled trial is now needed.

References

1. Zhang J, Zhu Y, Wang J, Hu H, Jin Y, Mao X, Zhang H, Ye Y, Xin X, Li D. Global burden and epidemiological prediction of polycystic ovary syndrome from 1990 to 2019: A systematic analysis from the Global Burden of Disease Study 2019. *PLoS One*. 2024 Jul 18;19(7):e0306991. doi:10.1371/journal.pone.0306991.
2. Lachenmeier DW, Walch SG, Padosch SA, Kröner LU. Absinthe–a review. *Crit Rev Food Sci Nutr.* 2006;46(5):365-77. doi:10.1080/10408690590957322. PMID: 16891209.

## 12.12. Association of metabolic syndrome with neuroimaging and cognitive outcomes in the UK Biobank

Qureshi D, Topiwala A, Al Abid SU, Allen NE, Kuźma E, Littlejohns TJ

*Diabetes Care*. 2024 Aug 1;47(8):1415-1423.
doi:10.2337/dc24-0537.

Brief summary: This observational study assessed the association between metabolic syndrome (MetS) and its components with structural neuroimaging outcomes and cognitive domains in 37,395 dementia-free adults from the UK Biobank. MetS was associated with poorer brain health, characterized by reduced brain volume, increased vascular pathology, and diminished cognitive function.

Comment: MetS is characterized by the cluster of central obesity, hypertension, dyslipidemia, and impaired glucose tolerance. It is well-established that insulin resistance plays a central role in both initiating and perpetuating MetS. Insulin also has critical functions in the brain, such as promoting neuron growth, modulating the release and uptake of catecholamines, and regulating the expression and distribution of gamma-aminobutyric acid (GABA), all of which contribute to maintaining cognitive function.[1] The presence of insulin receptors in the frontal cortex and hippocampus underscores its pivotal role in learning and memory. Therefore, insulin resistance-induced alterations in insulin signaling may accelerate brain aging, affect neural plasticity, and potentially lead to neurodegeneration.

This study performed neuroimaging and cognitive assessments in dementia-free adults with MetS compared to healthy individuals without MetS. Key findings revealed that MetS health was associated with reduced total brain volume and gray matter volume, which are essential for information processing, as well as increased white matter hyperintensities, often linked to aging, cognitive decline, and dementia. Having more MetS components correlated with reduced brain volume and increased white matter hyperintensities. Specifically, waist circumference and higher HbA1c levels were associated with lower total brain and gray matter volumes, while high blood pressure showed the strongest association with increased white matter hyperintensity volume.

Study participants with MetS performed worse on cognitive tests of working memory, information recall, processing speed, verbal and numerical reasoning, nonverbal reasoning, and executive functions related to planning and problem-solving.

These findings are crucial. Previous research showed that managing MetS components can improve brain health outcomes. For example, aggressive blood pressure management has been shown to slow the progression of white matter hyperintensities. Similarly, metformin as been linked to slower cognitive decline and reduced risk of dementia, while physical activity improves cognition in people with diabetes. Additionally, there is evidence that GLP-1R agonists may have neuroprotective effects.

References

1. Albar NY, Hassaballa H, Shikh H, Albar Y, Ibrahim AS, Mousa AH, Alshanberi AM, Elgebaly A, Bahbah EI. The interaction between insulin resistance and Alzheimer's disease: a review article. *Postgrad Med.* 2024 May;136(4):377-395. doi:10.1080/00325481.2024.2360887.

## 12.13. Influence of oral contraceptives on lipid profile and trajectories in healthy adolescents-data from the EVA-tyrol study

Staudt A, Kiechl SJ, Gande N, Hochmayr C, Bernar B, Stock K, Geiger R, Egger A, Griesmacher A, Knoflach M, Kiechl-Kohlendorfer U, Early Vascular Ageing (EVA) Study Group.

*J Adolesc Health*. 2024;75(3):479-486.
doi:10.1016/j.jadohealth.2024.04.017.

Brief summary: In a large cohort of healthy adolescents, oral contraceptive (OC) users showed an higher LDL and triglyceride blood levels than non-users. Similarly, lipid trajectories over time showed an increase in LDL and triglyceride levels.

Comment: Oral contraceptives (OCs) are widely used among young women. Studies in adults reported that OCs increase triglyceride levels, but effects on LDL levels were inconsistent. However, many of these studies were based on older OC formulations. The current study assessed the impact of OC use on lipid levels, BMI and blood pressure in youth.

Firstly, the authors performed a cross-sectional analysis of data from 317 adolescents OC users (mostly using monophasic combined OC) compared with 511 OC non-users. OC use was robustly associated with total and LDL-cholesterol as well as triglyceride levels, in models adjusted for age, socioeconomic status, BMI z-score, physical activity, and diet. Triglycerides levels were ∼48.6% higher in OC users. Increases in BMI and blood pressure were statistically significant, but the differences were mild and of doubtful clinical relevance.

Secondly, they performed a longitudinal analysis of 558 female adolescents, with an average 22 months duration of follow-up. Mean LDL increased by 15 mg/dL, and triglycerides by 36 mg/dL. Changes in BMI did not differ between those who initiated, continued, or discontinued OCs.

These changes are especially relevant for adolescents with other risk factors for dyslipidemia, such as severe obesity, T2D or other cardiovascular risk factors (1). When morbid obesity, severe hypertension, micro- or macrovascular disease, or multiple cardiovascular risk factors are present, it is advisable to prioritize use of progestin-only or nonhormonal contraceptive methods.

Reference

1. Merino PM, Codner E. Contraception for Adolescents and Young Women with Type 2 Diabetes-Specific Considerations. *Curr Diab Rep.* 2022 Feb;22(2):77-84.

## 12.14. Alirocumab in pediatric patients with heterozygous familial hypercholesterolemia: a randomized clinical trial

Santos RD, Wiegman A, Caprio S, Cariou B, Averna M, Poulouin Y, Scemama M, Manvelian G, Garon G, Daniels S

*JAMA Pediatr.* 2024 Mar 1;178(3):283-293. doi:10.1001/jamapediatrics.2023.6477.

Brief summary: This double-blind, randomized trial, showed that 2 dosing regimens of alirocumab, a human monoclonal antibody to proprotein convertase subtilisin kexin type 9 (PCSK9), reduced LDL-C in children as young as 8 years with heterozygous familial hypercholesterolemia inadequately controlled by statins. Efficacy was sustained over 2 years, and both regimens were generally well tolerated.

Comment: Heterozygous familial hypercholesterolemia (HeFH) is an autosomal dominant-inherited genetic disorder, with estimated prevalence 1:200–300 individuals. 20-year outcome data demonstrate lower rates of atherosclerotic cardiovascular disease (ASCVD) related events and death in individuals with HeFH treated with statins from childhood, compared to those who initiated statins in adulthood.[1] However, not all children respond to or tolerate statins.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is synthesized primarily in the liver, and enters the circulatory system, where it binds to hepatic LDL receptors, accelerating their degradation.[2] This process

reduces the capacity of the liver to remove LDL-C from the circulation. Individuals with PCSK9 loss-of-function mutations have low levels of LDL-C and low incidence of coronary heart disease. Based on this knowledge, a human monoclonal antibody targeting PCSK9 was developed and was approved by the U.S. FDA in 2015 as a second-line treatment for adults with HeFH or ASCVD whose cholesterol levels are uncontrolled by diet and maximally-tolerated dose of statins. It is now marketed with biweekly dosage of 75 mg (through a self-administered 1 mL pen) which can be up-titrated to 150 mg if necessary.

This study randomized 153 pediatric patients aged 8-17 years to receive injections of Alirocumab either 2-weekly or 4-weekly, while 52 were in the control group. Treatment for 24 weeks reduced LDL-C and other proatherogenic lipid parameters compared to placebo. The difference in % change from baseline was -43% for those treated 2-weekly, and -34% on 4-weekly. After the 24-week trial phase, all patients could receive open-label alirocumab for up to 80 weeks. Improvements in LDL levels were maintained at week 104. Alirocumab was well tolerated with no safety concerns. Based on these results, last year alirocumab use in children received approval in the EU and USA.

References

1. Peterson AL, McNeal CJ, Wilson DP. Prevention of Atherosclerotic Cardiovascular Disease in Children with Familial Hypercholesterolemia. *Curr Atheroscler Rep.* 2021 Aug 27;23(10):64. doi:10.1007/s11883-021-00959-8.
2. Yadav K, Sharma M, Ferdinand KC. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors: Present perspectives and future horizons. *Nutr Metab Cardiovasc Dis.* 2016 Oct;26(10):853-62. doi:10.1016/j.numecd.2016.05.006.

## 12.15. Cardiovascular outcomes in patients with homozygous familial hypercholesterolaemia on lipoprotein apheresis initiated during childhood: long-term follow-up of an international cohort from two registries

Reijman MD, Tromp TR, Hutten BA, Hovingh GK, Blom DJ, Catapano AL, Cuchel M, Dann EJ, Gallo A, Hudgins LC, Raal FJ, Ray KK, Sadiq F, Soran H, Groothoff JW, Wiegman A, Kusters DM
Homozygous Familial Hypercholesterolaemia International Clinical Collaborators (HICC); Children with Homozygous Hypercholesterolemia on Lipoprotein Apheresis: an International Registry (CHAIN) consortia.
*Lancet Child Adolesc Health*. 2024 Jul;8(7):491-499.
doi:10.1016/S2352-4642(24)00073-7.

Brief summary: Data from two large registries of children with homozygous familial hypercholesterolemia (HoFH) revealed that initiating lipoprotein apheresis in childhood, compared to pharmacotherapy, improves plasma LDL-C level and reduces cardiovascular death.

Comment: Homozygous familial hypercholesterolaemia (HoFH) is a rare inherited disorder, which results in extremely elevated low-density lipoprotein cholesterol (LDL-C) levels and premature atherosclerotic cardiovascular disease (ASCVD).

Lipoprotein apheresis is an extracorporeal lipid-lowering treatment that has been well established for 3 decades; it reduces serum LDL-C levels by > 70% per session. The current study assessed long-term cardiovascular outcomes associated with lipoprotein apheresis initiated in childhood or adolescence. It analysed the largest cohort to date with 250 children and adolescents: 125 treated with medication and 125 with apheresis.

Reductions in LDL-C concentrations were greater in the lipoprotein apheresis group (–55%) than medication group (–31%). However, given the very high initial LDL-C of 688 mg/dL, even this marked decrease could not prevent severe outcomes. Cardiovascular death was more common on medication-only than lipoprotein apheresis [8% *vs* 1%]; p = 0 010). However, the median age at coronary artery bypass grafting was lower in the lipoprotein apheresis group than medication-only group (15 0 or ·0 *vs* 30 5 years p = 0 037), and the prevalence and age of onset of other atherosclerotic cardiovascular disease were similar.

These findings are unsurprising given the low frequency of lipoprotein apheresis, which varied from twice per week to once per month, with biweekly sessions being the most common. Fewer than 10% of patients in each cohort reached target LDL-C concentrations, indicating that both therapies were generally sub-optimal.

A consensus statement on lipoprotein apheresis in children was recently published, based on current available evidence and expert opinions from around the world.[1] It covers indications, methods, vascular access, treatment

targets, monitoring of clinical efficacy, and side effects. By following this consensus and collecting data internationally, we will be able to learn valuable lessons to better understand the benefits and limitations of this treatment. In the meantime, you should review the effects of Evinacumab for treating HoFH.


Reference

1. Reijman MD, et al. Clinical practice recommendations on lipoprotein apheresis for children with homozygous familial hypercholesterolemia: an expert consensus statement from ERKNet and ESPN. *Atherosclerosis.* 2024 May;392:117525. doi:10.1016/j.atherosclerosis.2024.117525.


## 12.16. Evinacumab for pediatric patients with homozygous familial hypercholesterolemia


Wiegman A, Greber-Platzer S, Ali S, Reijman MD, Brinton EA, Charng MJ, Srinivasan S, Baker-Smith C, Baum S, Brothers JA, Hartz J, Moriarty PM, Mendell J, Bihorel S, Banerjee P, George RT, Hirshberg B, Pordy R

*Circulation*. 2024 Jan 30;149(5):343-353.

doi:10.1161/CIRCULATIONAHA.123.065529.


Homozygous familial hypercholesterolemia (HoFH) is a severe disorder caused by genetic mutations in *LDLR* (encoding the LDL receptor), *APOB* or *PCSK9*. LDL-C levels in HoFH are extremely elevated) > 400 mg/dL(even in utero, leading to cardiovascular events, and disability or death during childhood and adolescence. Conventional medications have minimal efficacy, since LDL-C levels cannot be reduced through upregulation of hepatic LDL receptors.

Angiopoietin-like 3 (ANGPTL3) is a hepatically secreted protein that acts as a potent inhibitor of lipoprotein lipase, the primary enzyme responsible for clearing triglyceride-rich lipoproteins from the circulation. Additionally, ANGPTL3 inhibits endothelial lipase, which aids the clearance of HDL from the bloodstream. Humans with loss-of-function variants in one copy of *ANGPTL3* have reduced serum LDL levels. Individuals with complete ANGPTL3 deficiency due to biallelic inactivating mutations have very low plasma lipid concentrations and tend to have less coronary atherosclerosis than those without ANGPTL3 deficiency

Evinacumab is a fully human monoclonal antibody that selectively binds to and inhibits ANGPTL3. When used alongside other lipid-lowering treatments, it reduced LDL-C by ∼50% in adults and adolescents aged 12+ years HoFH and was generally well-tolerated. Furthermore, reductions in plaque volumes were observed among adolescents after 6 months of evinacumab.

This is the first study to evaluate the efficacy and safety of evinacumab in children aged 5-11 years with HoFH. This 3-part, open-label study began with a 16-week phase assessing the safety, pharmacokinetics, and pharmacodynamics of a single intravenous dose of evinacumab (15 mg/kg) in 6 patients. It found comparable pharmacokinetics in children and adults. The second part was a 24-week, phase 3 study evaluating the efficacy, safety, and pharmacokinetics of evinacumab in 14 patients. The mean % change in LDL-C from baseline to week 24 was 48%. The third part is an ongoing 48-week, phase 3 extension, to evaluate long-term safety and efficacy.

The U.S. Food and Drug Administration (FDA) extended their approval of Evkeeza® (evinacumab-dgnb) as an adjunct to other lipid-lowering therapies to treat children aged 5-11 with HoFH.


References

1. Dingman R, Bihorel S, Gusarova V, Mendell J, Pordy R. Evinacumab: Mechanism of action, clinical, and translational science. *Clin Transl Sci.* 2024 Jun;17(6):e13836. doi:10.1111/cts.13836.


## 12.17. Association of health benefits and harms of Christmas dessert ingredients in recipes from The Great British Bake Off: umbrella review of umbrella reviews of meta-analyses of observational studies


Wallach JD, Gautam A, Ramachandran R, Ross JS

*BMJ*. 2023 Dec 20;383:e077166.

doi:10.1136/bmj-2023-077166.

Brief summary: Research published in the Christmas issue of The BMJ suggests that Christmas desserts from the television show, "The Great British Bake Off", are more likely to feature ingredients associated with a reduced risk of death or disease, rather than those that increase it.

Comment: Do not miss this article from the Christmas edition of The BMJ! The Great British Bake Off is the ultimate baking competition where passionate amateur bakers compete to be crowned the UK's Best Amateur Baker. The series showcases the challenges faced by competitors, young and old, from every background and every corner of Britain, as they strive to prove their baking prowess. Their recipes are published on the website.

There have long been concerns that the ingredients used to make modern Christmas desserts (eg, butter and sugar) are unhealthy. These authors evaluated the potential health benefits and harms of the ingredients used in various Christmas desserts. Instead of randomly selecting Christmas dessert recipes from cookbooks, all "Christmas" recipes listed on the official Great British Bake Off website were recorded, then categorised into 17 ingredient groups mostly likely to be evaluated in umbrella reviews. These included: alcohol; butter; chocolate; cheese and yogurt; coffee; eggs; food colouring, flavourings, and extracts; fruit; milk; nuts etc.

The authors conducted a literature search for umbrella reviews. They identified 46 umbrella reviews that included 363 unique associations between ingredients listed in the Christmas dessert recipes and risk of death or any disease. Overall, 41% of the summary associations between ingredient groups and the risk of death or disease were statistically significant. Of these, 74% suggested that the ingredient groups were associated with reduced risk of death or disease. The most common ingredient groups associated with reduced risk of death or disease were fruit, coffee and nuts. The 26% ingredient groups associated with increased death included alcohol and sugar.

The authors conclude that "this Christmas, if concerns about the limitations of observational nutrition research can be set aside, we are pleased to report that everyone can have their cake and eat it too."

# 13. Global Health for the Paediatric Endocrinologist

Diane Stafford, Emrullah Arslan
Division of Endocrinology and Diabetes, Stanford University School of Medicine, Stanford Medicine Children's Health Palo Alto, CA 94304, USA; Pediatric Endocrinology and Diabetes İzmir City Hospital Bayraklı, Türkiye

## Preface

This year's Global Health chapter focuses on improving diagnostic and therapeutic capabilities in pediatric endocrinology and diabetes in underserved populations. Understanding of variations in disease incidence and treatment practices, determining the feasibility of technology use, and working to establish clinical guidelines applicable to resource-limited settings will improve our ability to meet the needs and improve the health of those living in low and middle-income countries. International cooperation will continue to expand these abilities.

## Diabetes and Diabetes Technology

### 13.1. Feasibility of continuous glucose monitoring in patients with type 1 diabetes at two district hospitals in Neno, Malawi: a randomised controlled trial

Gomber A, Valeta F, Coates MM, Trujillo C, Ferrari G, Boti M, Kumwenda K, Mailosi B, Nakotwa D, Drown L, Wroe EB, Thapa A, Mithi V, Matanje B, Msekandiana A, Park PH, Kachimanga C, Bukhman G, Ruderman T, Adler AJ
*BMJ Open*. 2024 May 6;14(5):e075554.
PMID 38719319

Brief summary: This randomized-control study at two rural hospitals in Malawi investigated the feasibility of continuous glucose monitoring (CGM) to improve care of patients with Type 1 diabetes (T1D) in a low-income and limited literacy setting. CGM was feasible with potential benefits, but the authors highlight several challenges in implementing advanced diabetes care in such settings.

This trial 2:1 randomized 45 patients with T1D to Dexcom G6 CGM (n = 30) or usual care (UC) using a blood glucose meter (n = 15) for 3 months. Providers were trained to review downloads and logbook data, make dose adjustments and recommend lifestyle change at follow up visits scheduled monthly. Those in the CGM arm were seen more often for sensor changes. Baseline characteristics of both groups were similar. WHO Quality of Life Brief Version (WHOQOL-BREF) and HbA1c were primary outcomes.

Sensors were used 68% of the time in the CGM group. Inability to change sensors at home led to more visits in this group. UC participants brought logbooks to visits 75% of the time, but with glucose readings only 51.3% of the time. More insulin adjustments were made in the CGM arm (1.2 per person) than the UC arm (0.2 per person). More recommended lifestyle changes were made in the CGM arm (0.4 per person) than the UC arm (0.2). In the CGM arm, the average time in range (TIR) was 30.8% in week 1 and 38.7% in week 10. CGM was associated with a 1.1% lower HbA1c vs UC (adjusted for baseline HbA1c and covariates), but this trend was not statistically significant. Similarly, quality of life was improved across all domains, but was not significantly different between groups. The most significant issue for utilization was limited digital literacy of patients to use CGM devices independently.

CGM was feasible and appropriate in this rural setting, but challenges with sensor changes and digital literacy need addressing. The study highlights the potential of CGM to improve diabetes management in low-resource settings but calls for further research, particularly on newer, more user-friendly CGM models.

Reference

1. Battelino T, Alexander CM, Amiel SA, et al. Continuous glucose monitoring and metrics for clinical trials: an international consensus statement. *Lancet Diabetes Endocrinol* 2023;11:42–57.

## 13.2. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the global burden of disease study 2021

GBD 2021 Diabetes Collaborators.

*Lancet*. 2023 Jul 15;402(10397):203-234.
doi: 10.1016/S0140-6736(23)01301-6. PMID: 37356446.

Brief summary: This systematic analysis examined the prevalence of type 1 diabetes (T1D) and type 2 diabetes (T2D) burden from 1990 to the present, in relation to location, age, and gender, and made projections to the year 2050. It reports that the prevalence of T2D will continue to rise, with rising obesity being the primary factor.

According to the International Diabetes Federation (IDF), there were 537 million people with diabetes worldwide in 2021, and the cost to the global economy was 966 billion US dollars (1). This study ascertained age, gender, and location-specific rates of diabetes types in 204 countries and regions, and quantified 16 risk factors for T2D, using the Global Burden of Diseases, Injuries, and Risk Factors research (GBD) database.

The prevalence of diabetes was estimated to be 529 million individuals (6.1%). The prevalence is $>$ 10% in 18 countries and 15 regions, and highest in North Africa, the Middle East, and Oceania. T2D accounts for 96% of diabetes cases, but is $<$ 90% in Australia (86.4%) and Western Europe (89.3%). The combined impact of diabetes in 2021 was estimated to be 79.2 million disability-adjusted life-years (DALYs), with 37.8 million years of life lost (YLLs) and 41.4 million years lived with disability (YLDs). High BMI was the primary risk factor for T2D, responsible for 52.2% of the DALYs due to diabetes. By 2050, there will be 1.31 billion individuals diagnosed with diabetes. Rising obesity will account for 49.6% of this increase, and demographic changes (e.g. population size and age) for 50.6%.

These findings highlight obesity as the most significant risk factor for the increasing rates of diabetes. It is evident that an immediate action plan is required to combat obesity. Furthermore, in order to mitigate the adverse consequences of diabetes complications on the global economy and the quality of life of individuals, it is imperative to develop novel strategies that address the social and logistical obstacles that impede access to medical care and treatment.

Reference

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract.* 2022 Jan;183:109119. Erratum in: Diabetes Res Clin Pract. 2023 Oct;204:110945.

## 13.3. Celebrating the data from 100,000 real-world users of the MiniMed™ 780G system in Europe, Middle East, and Africa collected over 3 years: from data to clinical evidence

Choudhary P, Arrieta A, van den Heuvel T, Castañeda J, Smaniotto V, Cohen O

*Diabetes Technol Ther.* 2024 Mar;26(S3):32-37.
doi: 10.1089/dia.2023.0433. PMID 38377326.

Brief summary: This retrospective study analyzed data from real-world users of the MiniMed™ 780G System. Hybrid closed-loop algorithms have proven to be successful in achieving glycemic goals in people with diabetes. This performance has been consistently observed in various age groups and geographic areas.

After the introduction of the first commercial automated insulin systems in 2016, the number and variety of these devices expanded rapidly (1). This study examined real-world data from 101,629 users of the MiniMed™ 780G

System in 34 countries spanning Europe, the Middle East, and Africa between 2021 and 2023. It comparing utilization rates among ideal settings, age categories, and regional/country subgroups. The mean time in range (TIR) for all users was 72.3%, the glycemic management indicator (GMI) was 7%, time < 70 mg/dL (TBR70) was 2.0%, and time < 54 mg/dL (TBR54) was 0.4%. In addition, the percentage of individuals who achieved worldwide glycemic objectives was 59.6% for GMI < 7%, 62.5% for TIR > 70%, 88.4% for TBR < 4% for 70% of the time, and 90.0% for TBR of < 1% for 54% of the time. Performance was better among users in an ideal situation; 86.3% had TIR > 70%. The average TIR in the longitudinal cohort remained > 73.3% for 12 months, while TBR70 and TBR54 fell below international targets during this period. The mean TIR was 69.9% in children aged > = 15 years, who constituted 22% of the study group, and 76.8% in adults aged 56+ years, who constituted 13% of the group.

This study provides compelling evidence of the efficacy of the MiniMed™ 780G System in attaining glycemic goals among various age groups and geographical locations, based on extensive real-world data. Effort was made to overcome the study's inability to compare diabetes subtypes, by categorizing participants by age. Nevertheless, the absence of a thorough analysis of the progressive decline in GMI, TIR, and time above range over the course of 12 months in the longitudinal sample is a notable limitation.

Reference

1. Da Silva J, Bosi E, Jendle J, Arrieta A, Castaneda J, Grossman B, Cordero TL, Shin J, Cohen O. Real-world performance of the MiniMed™ 670G system in Europe. *Diabetes Obes Metab.* 2021 Aug;23(8):1942-1949.

## 13.4. Diabetes care and outcomes of pediatric refugees and migrants from Ukraine and Syria/Afghanistan with type 1 diabetes in German-speaking countries

Auzanneau M, Reinauer C, Ziegler J, Golembowski S, de Beaufort C, Schöttler H, Hahn E, Mirza J, Galler A, Wurm M, Holl RW
*Front Endocrinol (Lausanne)*. 2024 Jun 7;15:1403684.
doi: 10.3389/fendo.2024.1403684. PMID: 38919493.

Brief summary: This multicenter, observational study compared the treatment of diabetes and outcomes between 2 distinct refugee communities in German-speaking countries and among native children in the same countries. The use of advanced diabetes technologies, such as insulin pumps or automated insulin delivery (AID) systems, is restricted by the refugee status. Additionally, parameters such as HbA1c and body mass index (BMI) have been identified to differ by country of origin of refugees.

There is a scarcity of data on the management of type 1 diabetes (T1D) in war refugee children, who are particularly susceptible to health issues. This study used data from the German/Austrian/Luxembourgian/Swiss diabetes prospective follow-up registry (DPV). The authors compared diabetes treatment and outcomes in the host country of children with Type 1 Diabetes (T1D) from Ukraine (U), refugees from Syria/Afghanistan (S/A), and those who never migrated (Controls). Compared to controls, both refugee groups had lower use insulin pumps (U: 24.9%, S/A: 19.9%, Controls: 59.1%) and AID (U: 8.8%, S/A: 4.7%, Controls: 24.4). Continuous glucose monitor use was considerably lower among S/A refugees (U: 81.6%, S/A: 54.5%, Controls: 85.5%). BMI SDS was relatively higher among S/A refugees and controls (U: 0.02, S/A: 0.24, Controls: 0.22), and S/A refugees had a higher HbA1c (U: 7.22%, S/A: 7.59%, Controls: 7.2%). The prevalence of severe hypoglycemia was elevated among S/A refugees.

These findings highlight the need for a more comprehensive examination of the issue of immigrants in order to improve their healthcare, which compares two distinct groups of refugees from various origins with local individuals living with T1D in the host country. The countries of origin and the diets of immigrant children with T1D, as well as language education and health literacy, should be taken into account.

References

1. Moore TH, Dawson S, Wheeler J, Hamilton-Shield J, Barrett TG, Redwood S, et al. Views of children with diabetes from underserved communities, and their families on diabetes, glycaemic control and healthcare provision: A qualitative evidence synthesis. *Diabetes Med*. (2023) 40:e15197. doi: 10.1111/dme.15197
2. Neuman V, Vavra D, Drnkova L, Pruhova S, Plachy L, Kolouskova S, et al. Introduction of continuous glucose monitoring (CGM) is a key factor in decreasing HbA1c in war refugee children with type 1 diabetes. *Diabetes Res Clin Pract*. (2024) 208:111118. doi: 10.1016/j.diabres.2024.111118.

## 13.5. Patient-led innovation and global health justice: Open-source digital health technology for type 1 diabetes care

Jansky B, Hendl T, Nocanda AZ

*Bioethics*. 2024 Jul;38(6):511-528.
doi: 10.1111/bioe.13205. PMID: 37830740.

Brief summary: This article evaluates the social conditions and global inequalities that shape patient-led innovation in the context of type 1 diabetes (T1D). The multidisciplinary team used an empirical sociological approach combined with normative ethical analyses. They conclude that open-source, patient-led innovation should be developed with a proactive strategy primarily targeting groups experiencing inequalities in access to health services.

The access of societies to the right to health is influenced by sociopolitical and economic inequalities, as multiple studies have shown (1,2,3). This study examines the influence of open source patient-led innovations in the context of T1D, which are characterized as "bottom-up" due to their patient-led nature, as opposed to traditional, on the elimination of social and health inequalities. The open-source community played a significant role in promoting awareness of the persistent burden associated with living with T1D and the issue of slow innovation cycles in T1D technology development. This paved the way for the more rapid development of commercial health technologies. The community is still in a precarious situation and is at significant risk, despite these positive effects. In a situation such as T1D, where the individual affected by the disease primarily assumes responsibility for the disease's management, this precariousness is indicative of the increasing personal responsibility for health. One of the most significant deficiencies of patient-led innovation is that the population groups with the least access to healthcare and their health requirements are not included in "bottom-up" innovation discussions. For this reason, innovation under patient leadership continues to be embedded within many socio-political and economic inequalities while disrupting the power asymmetry held by physicians and medical technology manufacturers in traditional hierarchies.

This study emphasizes the rationale behind utilizing patient-led innovation in T1D and predicts its increasing prevalence in the future. It suggests that healthcare professionals should prioritize the use and significance of T1D open source technologies and their integration into the healthcare system.

References

1. Marmot, M. G., & Wilkinson, R. G. Global justice and the social determinants of health. *Ethics & International Affairs*, 24(2), 119–130; Chung, R. (2021).
2. Hendl, T., Jansky, B., & Wild, V. From design to data handling: Why mHealth needsa feminist perspective. In J. Loh & M. Coeckelbergh (Eds.), Feminist philosophy of technology(pp. 77–103). J.B. Metzler; Liao, S. Y., & Carbonell, V. (2022).
3. ibid; Criado-Perez, C. (2019). Invisible women: Exposing data bias in a world designed for men.Penguin

# Endocrinology

## 13.6. Identification of southern Taiwan genetic variants in thyroid dyshormonogenesis through whole-exome sequencing

Tsai CC, Chang YM, Chou YY, Chen SY, Pan YW, Tsai MC

*Kaohsiung J Med Sci*. 2024 Jun 25.
doi: 10.1002/kjm2.12871. PMID: 38923290.

Brief summary: This cohort study identified genetic variants associated with thyroid dyshormonogenesis as a cause of congenital hypothyroidism in Southern Taiwan. It reviewed 876 CH patients diagnosed between 2011 and 2022 and examined the genetic etiology in 47 cases of TDH using whole-exome sequencing (WES).

The etiology of congenital hypothyroidism (CH) can be attributed to either thyroid dysgenesis (TD) or thyroid dyshormonogenesis (TDH) with TD usually identified in 75-85% of cases. Several studies have shown significant variation in the incidence of TDH that may be due to demographic shifts and genetic "hot spots". The investigators utilized the current technology for whole-exome sequencing to determine the genotype of a large

cohort of patients with TDH seen in a single center in Taiwan. 876 cases of (CH) were identified at the National Cheng Kung University Hospital from 2011 to 2022. 121 cases were classified as permanent CH based on requirement for levothyroxine for a minimum of 3 years after birth and, of these, 47 (40%) were classified as TDH based on imaging-confirmed presence of bilateral normal thyroid structures on ultrasound or Tc99m scan. Whole-exome sequencing was performed on 45 patients, with causative variants identified in 32 patients (71.1%). The most common mutations were in genes for DUOX2 (15 cases), TG (8 cases), TSHR (7 cases), TPO (5 cases), and DUOXA2 (1 case). Four recurrent mutations were identified and four novel variants discovered. While previous studies have identified mutations in TG as the most common cause of TDH in Western populations, this study support previous identification of DUOX2 as more common in Asian countries. The DUOX2 R1110Q mutation accounted for 13.3% in this study, higher than the 2.2% in mainland China previously reported, suggesting regional differences in the genetic variations that may be related to demographic shifts.

These findings underscore the utility of WES in providing a molecular diagnosis for cases of TDH. It highlights the diversity of genetic etiologies in Taiwan and the potential regional differences in genetic mutations. Wider routine use of WES would allow for a more detailed analysis of the genetic causes of TDH.

References

1. Stoupa A, Kariyawasam D, Polak M, Carré A. Genetics of congenital hypothyroidism: modern concepts. *Pediatr Investig*. 2022;6(2): 123–34.
2. Kara C, Mammadova J, Abur Ü, Gumuskaptan C, _ Izci Güllü E, Dagdemir A, et al. Genetic testing can change diagnosis and treatment in children with congenital hypothyroidism. *Eur Thyroid J.* 2023;12(3):e220212.

## 13.7. Vitamin D supplements for fracture prevention in schoolchildren in Mongolia: analysis of secondary outcomes from a multicentre, double-blind, randomised, placebo-controlled trial

Ganmaa D, Khudyakov P, Buyanjargal U, Tserenkhuu E, Erdenenbaatar S, Achtai CE, Yansanjav N, Delgererekh B, Ankhbat M, Tsendjav E, Ochirbat B, Jargalsaikhan B, Enkhmaa D, Martineau AR

*Lancet Diabetes Endocrinol*. 2024 Jan;12(1):29-38.
PMID: 38048799.

Brief summary: This multicenter, double-blind, randomized, placebo-controlled trial evaluated the effects of vitamin D supplementation (14,000 IU D3 weekly for 3 years) on fracture risk and other bone health indicators in 8,851 schoolchildren aged 6–13 years in 18 public schools in Mongolia, where vitamin D deficiency is highly prevalent with a high fracture burden.

Vitamin D3 doses were given during weekly face-to-face visits in schools. Families completed a questionnaire detailing socioeconomic, lifestyle and dietary factors that may influence vitamin D status, and intake of foods contributing to dietary calcium. There were similar numbers in the vitamin D group (4,176) and the placebo group (4,172) for the fracture and safety analyses.

Widespread vitamin D deficiency was noted at baseline. 95.5% of children had a serum 25-hydroxyvitamin D [25(OH)D] concentrations $<$ 50 nmol/L; mean baseline was 29.6 nmol/L in both groups. Over the 3-year follow-up, 268 children (6.4%) in the vitamin D group and 253 (6.1%) on placebo reported one or more fractures. There was no difference in fracture risk between groups (adjusted risk ratio 1.10). A sub-study used a radial quantitative ultrasound measure of bone density and bone strength (radial speed of sound) at baseline, 1, 2 and 3 years. There was no difference in bone strength between groups. A second sub-study (50 children per group) performed biochemical analysis and found no effect modification by sex, baseline vitamin D concentration, or calcium intake on fracture risk or SOS.

Weekly vitamin D supplementation with apparently appropriate doses (equal to 2000 IU daily) increased serum vitamin D levels and suppressed PTH, but did not reduce the risk of fractures in this population. These negative findings, particularly in an area with high rates of vitamin D deficiency, indicate that routine supplementation of vitamin D may not be an effective public health strategy.

References

1. Wu F, Fuleihan GE, Cai G, et al. Vitamin D supplementation for improving bone density in vitamin D-deficient children and adolescents: systematic review and individual participant data meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2023; 118: 498-506.
2. GBD 2019 Fracture Collaborators. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev* 2021; 2: e580-92.

## 13.8. Experiences and challenges with congenital hypothyroidism newborn screening in Indonesia: a national cross-sectional survey

Pulungan AB, Puteri HA, Faizi M, Hofman PL, Utari A, Chanoine JP

*Int J Neonatal Screen*. 2024 Jan 19;10(1):8.
doi: 10.3390/ijns10010008. PMID: 38390972.

Brief summary: This cross-sectional survey of healthcare professionals and administrators in Indonesia investigated the many challenges raised during implementation of a national newborn screening program for congenital hypothyroidism (CH).

Despite initiation of Newborn Screening (NBS) for CH in Indonesia between 2000 and 2010 and expansion more recently, only 2.3% of newborns were screened in 2022. This national survey aimed to identify barriers to wider uptake of CH NBS in Indonesia, receiving responses from 423 healthcare professionals who participated in a webinar focused on improving pediatric endocrine care in low and middle-income countries (LMICs). Questions focused on the challenges experienced and attitudes towards CH NBS.

One third of respondents reported that their facilities had not been able to screen all newborns. The major challenges reported include early discharge of newborns (less than 24 hours after birth) (38.3%), limited availability of filter paper (35.9%), and technical difficulties in sample collection and processing (42.6%). A significant number of parents refuse screening (39.2%) due to fear or a lack of understanding of the importance. The majority of healthcare professionals (96.5%) believe that parents lack sufficient knowledge about CH NBS, impacting the acceptance and success of the program. Only 38.5% of healthcare professionals reported receiving formal training in CH NBS. Most healthcare professionals (87%) felt confident in educating parents about CH NBS, although fewer (69.5%) felt they had sufficient understanding of the CH NBS system in Indonesia. The majority felt CH NBS was beneficial and supported extension to other congenital conditions.

This study highlights significant challenges in achieving universal CH NBS coverage in Indonesia, including logistical, educational, and infrastructural barriers. It is encouraging that there has been a significant increase in government commitment with unpublished data indicating national coverage of 15.53% as of October 2023. The implementation of NBS programs in LMICs is complicated by factors such as public health priorities. The authors emphasize the need for better training for healthcare professionals, better parental education, and enhanced logistical support to increase the program's effectiveness and coverage across the country.

References

1. Kopel, J.A global perspective on newborn congenital hypothyroidism screening. *Baylor Univ. Med. Cent. Proc.* 2020, 33, 137–139.
2. LaFranchi, S.H. Worldwide coverage of newborn screening for congenital hypothyroidism-a public health challenge. *US Endocrinol*. 2014, 10, 115–116.

## 13.9. 17α Hydroxylase/17,20 lyase deficiency: clinical features and genetic insights from a large Turkey cohort

Siklar Z, Camtosun E, Bolu S, Yildiz M, Akinci A, Bas F, Dündar İ, Bestas A, Ünal E, Kocaay P, Guran T, Buyukyilmaz G, Ugurlu AK, Tosun BG, Turan I, Kurnaz E, Yuksel B, Turkkahraman D, Cayir A, Celmeli G, Gonc EN, Eklioğlu BS, Cetinkaya S, Yilmaz SK, Atabek ME, Buyukinan M, Arslan E, Mengen E, Cakir EDP, Karaoglan M, Hatipoglu N, Orbak Z, Ucar A, Akyurek N, Akbas ED, Isik E, Kaygusuz SB, Sutcu ZK, Seymen G, Berberoglu M

*Endocrine*. 2024 Jul 17.
doi: 10.1007/s12020-024-03962-6. PMID: 39020240.

Brief summary: This retrospective cohort study analyzed data from 97 cases of 17α hydroxylase/17,20 lyase deficiency identified in Turkey. It focused on diagnostic testing, laboratory characteristics, long-term outcomes and therapeutic management.

This nationwide study focuses on 17α-Hydroxylase/17,20-Lyase deficiency (17OHD), a rare form of congenital adrenal hyperplasia (CAH) caused by mutations in the CYP17A1 gene. The research aims to evaluate the clinical, biochemical, and genetic characteristics of 97 patients with 17OHD in Turkey, one of the largest studies of its kind.

Patients were recruited from 32 pediatric endocrinology centers across Turkey. Retrospective data included physical examination, hormone assays, genetic findings, and follow-up information. All cases had genetically confirmed *CYP17A1* gene mutations. The 97 cases identified came from 78 families. 59 cases (60.8%) had 46,XY karyotype and 38 (39.1%) had 46,XX karyotype. Most patients (94%) were raised as females, and many presented with primary amenorrhea (n = 46) or delayed puberty (n = 45). Hypertension was detected in 65% of patients, and 34% had hypokalemia. There was a high rate of consanguinity (affecting 87 patients in 78 families).

The most common mutation was a homozygous deletion in Exon 1–6 of the CYP17A1 gene, found in 42 cases (45.8%). Two different previously reported large deletions were found in 3 siblings. Other mutations, mainly point mutations, were also identified and generally concentrated in Exon 1 and Exon 6. No significant genotype-phenotype correlation was found.

These findings highlight the importance of early diagnosis and management of 17OHD, particularly in populations with high rates of consanguinity. Hypertension and hypokalemia are key indicators for early diagnosis with the most common presentation being an adolescent girl without secondary sexual characteristics or menses and low-renin hypertension. Genetic testing should prioritize the detection of large deletions in the CYP17A1 gene. This research provides valuable insights into the clinical management and genetic landscape of 17OHD and underscores the need for continued research into the genotype-phenotype correlations of this rare condition.

References

1. R.J. Auchus, Steroid 17-hydroxylase and 17, 20-lyase deficiencies, genetic and pharmacologic. *J. Steroid Biochem. Mol. Biol.* (2017).
2. Y. Xu, S. Jiang, Z. Yan, Y. Niu, W. Du, B. Liu, B. Han, X. Liu, S. Zhao, H. Song, Y. Kuang, J. Qiao, Phenotypic heterogeneity and fertility potential of patients with 17-hydroxylase/17,20-lyase deficiency. *J Clin Endocrinol Metab.* (2022).

## 13.10. Lessons learned from the real-world diagnosis and management of hereditary hypophosphatemic rickets

Chaturvedi D, Mehasi TE, Benbrahim A, ElDeeb L, Deeb A
*Bone Rep*. 2024 Mar 21;21:101753.
doi: 10.1016/j.bonr.2024.101753. PMID: 39011543.

Brief summary: This case-series describes the clinical characteristics and genetics of 8 patients with hereditary hypophosphatemic rickets (HHR). It highlights the challenges faced in diagnosis and management.

Hypophosphatemic rickets (HHR) is often underdiagnosed or misdiagnosed, leading to delays in proper treatment. Misdiagnosis can result in inappropriate therapies and worsening of the condition. The cases presented highlight the importance of comprehensive genetic testing for accurate diagnosis, as traditional diagnostic methods may not detect all variants.

HHR is caused by mutations in several genes, with *PHEX* being the most common. However, the expressivity of these mutations can vary, even within the same family, complicating diagnosis and management. Conventional treatments, primarily phosphate supplements and active vitamin D analogs, are often insufficient in managing HHR. Poor compliance due to side effects and the burden of frequent dosing is common. Burosumab, a new treatment option that targets FGF23, has shown promise in improving outcomes, but access to this therapy is not equitable, with many patients unable to afford it.

The authors present 8 clinical vignettes of patients with HHR encountered in a tertiary pediatric endocrinology centre in the United Arab Emirates. It describes clinical features, genetics, and management of 4 cases of X-linked hypophosphatemia (*PHEX* mutations), one each of autosomal recessive HHR (*DMP1* mutation) and autosomal recessive vitamin D-dependent rickets type 1A (*CYP27B1* mutation), and two cases of distal renal tubular acidosis with *FOXI1* mutation-associated HHR. These cases emphasize the need for individualized treatment plans and the potential benefits of newer therapies like Burosumab.

These cases underscore the complexities of managing HHR and advocates for improved diagnostic and treatment protocols to ensure better patient care. There is a call for better awareness, earlier genetic testing, and more consistent access to effective treatments like burosumab. Equitable access to such therapies is crucial to improving patient outcomes.

References

1. Gonzalez-Lamuno, D., 2020. Hypophosphataemic rickets: diagnosis algorithm-how not to make a mistake. *Adv. Ther.* 37 (Suppl. 2), 95-10495–104. 10.1007/s12325-019-01184-1.
2. Haffner, D., Emma, F., Eastwood, D.M., Duplan, M.B., Bacchetta, J., Schnabel, D., et al., 2019. Clinical practice recommendations for the diagnosis and management of X-linked hypophosphataemia. *Nat. Rev. Nephrol.* 15 (7), 435–455. 10.1038/s41581-019-0152-5.

## 13.11. GRADE-ADOLOPMENT of hyperthyroidism treatment guidelines for a Pakistani context

Martins RS, Nadeem S, Aziz A, Raja S, Pervez A, Islam N, Ahmed A, Sheikh A, Furqan S, Ram N, Rizwan A, Rizvi NA, Mustafa MA, Aamdani SS, Ayub B, Masood MQ

*BMC Endocr Disord*. 2024 Mar 21;24(1):41.
doi: 10.1186/s12902-023-01493-1. PMID: 38509509.

Brief summary: This process study describes the adaptation of previously established clinical practice guidelines for hyperthyroidism to better suit the local healthcare context in Pakistan. Adaptation utilized the GRADE-ADOLOPMENT method.

Hyperthyroidism is more prevalent in Pakistan (2.9%) than in the US and Europe. Pakistan faces unique challenges due to its diverse geography, dietary habits, and healthcare financing issues. Local guidelines are needed because imported guidelines from high-income countries may not fit the local context due to differences in disease epidemiology, healthcare infrastructure, and financial constraints.

The GRADE-ADOLOPMENT method utilized for this project was developed by GRADE (Grading of Recommendations Assessment, Development, and Evaluation) and is a globally accepted and implemented process for development of evidence-based clinical practice guideline. Adolopment describes a combination of adoption, adaptation (contextual modifications), and de novo development. The 2016 American Thyroid Association Guidelines served as the source for guideline development in Pakistan. Out of 124 recommendations in the source guideline, 71 were adopted, 49 excluded, and 4 were adapted. Adaptations included simplifying the use of laboratory tests to reduce patient costs, reflecting the resource constraints in Pakistan.

There were several key adaptations established generally in order to decrease costs, simplify follow-up or due to lack of availability. The process highlighted challenges related to resource constraints, stakeholder involvement, and resistance to change. Solutions included involving more stakeholders, emphasizing the need for local guidelines, and considering the diverse healthcare landscape of Pakistan.

These adapted guidelines provide context-specific, cost-effective recommendations for the management of hyperthyroidism in Pakistan. The authors emphasize the importance of guidelines that are not only evidence-based but also tailored to the local context, ensuring they are both practical and applicable in a resource-limited setting. Future research is needed to validate the effectiveness and feasibility of these adapted guidelines across the country.

References

1. Schünemann HJ, Wiercioch W, Brozek J, Etxeandia-Ikobaltzeta I, Mustafa RA, Manja V, et al. GRADE evidence to decision (EtD) frameworks for adoption, adaptation, and de novo development of trustworthy recommendations: GRADE-ADOLOPMENT. *J Clin Epidemiol.* 2017;81:101–10.
2. Ross DS, Burch HB, Cooper DS, Carol G, Luiza M, Rivkees SA, et al. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. *Thyroid.* 2016;26(10):1343–1421.

## 13.12. High frequency of transient congenital hypothyroidism among infants referred for suspected congenital hypothyroidism from the Turkish National screening program: thyroxine dose may guide the prediction of transients

Özer Y, Anık A, Sayılı U, Tercan U, Deveci Sevim R, Güneş S, Buhur Pirimoğlu M, Elmaoğulları S, Dündar I, Ökdemir D, Besci Ö, Jalilova A, Çiçek D, Singin B, Ulu ŞE, Turan H, Albayrak S, Kocabey Sütçü Z, Eklioğlu BS, Eren E, Çetinkaya S, Savaş-Erdeve Ş, Esen I, Demir K, Darcan Ş, Hatipoğlu N, Parlak M, Dursun F, Şıklar Z, Berberoğlu M, Keskin M, Orbak Z, Tezel B, Yürüker E, Keskinkılıç B, Kara F, Erginöz E, Darendeliler F, Evliyaoğlu O

*J Endocrinol Invest*. 2024; 47(9):2213-2224.
doi: 10.1007/s40618-024-02348-9. PMID: 38546931.

Brief summary: This retrospective, multicenter study examined patients who were diagnosed with congenital hypothyroidism (CH) through the newborn screening (NSP) program in Turkey. It assessed the prevalence of temporary congenital hypothyroidism (TCH), examined the causes of permanent congenital hypothyroidism (PCH), and identified laboratory and clinical indicators to distinguish TCH and PCH.

Congenital hypothyroidism (CH), the most prevalent endocrine disorder in children, affects around 1:2000 individuals in Western populations [1]. The incidence in Turkey is 1:2183 [2]. This study included 239 children, born in 2015 and were diagnosed with CH via NSP. They were tracked for a minimum of 6 years at 17 different centers. PCH was identified in 46.4% of the patients, of whom 39.6% had dysgenesis and 60.4% had dishormonogenesis. Identified predictive parameters for TCH were low serum TSH $<$ 100 μIU/mL at diagnosis (specificity: 81.5%, sensitivity: 41.3%), low TSH $<$ 45 μIU/mL in the first heel blood sample taken for NSP (specificity: 93.1%, sensitivity: 45.5%), and low LT4 dose $<$ 2 μg/kg/day at the treatment cessation period (specificity: 94.5%, sensitivity: 55.7%). In patients with eutopic glands, the only predictive parameter was low LT4 dose $<$1.1 μg/kg/day at treatment cessation (sensitivity: 84.7%, specificity: 40.4%).

The study's limitations are the absence of perchlorate discharge testing or genetic studies in elucidating the etiology of PCH. However, as there is still ongoing discussion over when exactly to stop LT4 medication, it is helpful to highlight the predictive value of TSH levels in the initial heel puncture sample and the LT4 dose when treatment is being discontinued. Lowering the LT4 dose threshold for identifying TCH in patients with eutopic glands enhances the existing evidence.

References

1. Mitchell ML, Hsu HW, Sahai I; Massachusetts Pediatric Endocrine Work Group. The increased incidence of congenital hypothyroidism: fact or fancy? *Clin Endocrinol (Oxf).* 2011 Dec;75(6):806-10. doi: 10.1111/j.1365-2265.2011.04128.x. PMID: 21623857.
2. Ataş B, Altunhan H, Ata E, Müsevitoğlu A. Frequency of congenital hypothyroidism in neonates in the Konya region, Turkey. *J Pediatr Endocrinol Metab.* 2011;24(3-4): 139-40. doi: 10.1515/jpem.2011.078. PMID: 21648280.

# Identifying Health Disparities and Improving Access to Healthcare

## 13.13. Refocusing the World Health Organization's model list of essential medicines on the needs of low and middle income countries

Wirtz VJ, Gray AL, Sharma S, Sun J, Hogerzeil HV

*BMJ*. 2024 Apr 16;385:e077776.
doi: 10.1136/bmj-2023-077776. PMID: 38626944.

Brief summary: This commentary argues to refocus the WHO Model List of Essential Medicines on the needs of low and middle-income countries, reiterating the original goals of the process to promote equitable access to medicine and improve health globally.

The World Health Organization (WHO) Model List of Essential Medicines, first published in 1977, is designed to promote equitable access to essential medicines that address the priority health needs of populations. It includes medicines selected based on public health relevance, evidence of efficacy and safety, and cost-effectiveness. The list has become a critical tool in guiding national medicine policies and universal health coverage and Is now incorporate into the national essential medicine list of over 150 countries.

The model list is specifically intended to present evidence-based comparisons of safety, efficacy and cost effectiveness, focusing on value for money and supporting procurement and reimbursement decision. In the last decade, there has been a rise in applications to include expensive and highly specialized medicines, especially those requiring advanced healthcare infrastructure with upper-middle and high-income countries adapting the essential medicine concept to define reimbursement. This trend challenges the relevance of the list for low and middle-income countries (LMICs), which may not have the resources to provide these medicines sustainably. Medicines included in the list should be those that offer high public health value, particularly in LMICs. WHO should prioritize medicines that are of high public health relevance to LMICs and refine its guidelines for submitting price and cost-effectiveness data, ensuring they are applicable to LMICs. The inclusion of medicines for rare diseases or those requiring sophisticated diagnostic capabilities should be reconsidered unless these medicines are essential and can be sustainably provided in these settings.

The authors advocate for a realignment of the WHO Model List of Essential Medicines to better serve the needs of LMICs, emphasizing the importance of equity, efficiency, and relevance in medicine selection. The WHO is encouraged to maintain its role in providing global guidance while ensuring that its recommendations are practical and actionable for countries with limited resources.

References

1. World Health Organization. The selection of essential drugs, including the first Model List of Essential Drugs. 1977. Technical Report Series.
2. Perehudoff K, Toebes B, Hogerzeil H. A human rights-based approach to the reimbursement of expensive medicines. *Bull World Health Organ* 2016;94:-6. doi: 10.2471/BLT.15.166371 pmid: 27994287

## 13.14. Physical late effects of treatment among survivors of childhood cancer in low- and middle-income countries: a systematic review

Wong KA, Moskalewicz A, Nathan PC, Gupta S, Denburg A

*J Cancer Surviv.* 2024 Jan 6.
doi: 10.1007/s11764-023-01517-8. Epub ahead of print. PMID: 38183576.

Brief summary: This systematic review investigated the physical residual effects of treatment on pediatric cancer survivors in low- and middle-income countries (LMICs), a topic with substantial information voids. Selection bias, limited sample sizes, and wide differences in the definition of long-term treatment effects were identified and these limited the ability to perform meta-analyses.

In high-income countries (HICs), the 5-year survival rates following pediatric cancers have surpassed 80%. Nevertheless, numerous studies conducted in HICs have demonstrated that these patients are at risk for subsequent malignant neoplasms (SMN), cardiovascular, or endocrinological complications, contingent upon the type of primary malignancy, the treatment administered, and the time since diagnosis[1]. There are insufficient data in LMICs to comment on this issue. To address the knowledge deficit on this topic in LMICs, the authors compiled 16 articles and 5 conference abstracts by scanning publications with a median follow-up period of at least 5 years after cancer, published before November 2022, across 5 search engines. In the survivors, 0-11% had SMN, 1-16% had cardiovascular complications, 1-46% had obesity, 4-62% had dyslipidemia, 1-3% had diabetes, 5-26% had impaired growth, 2-49% had hypothyroidism, 3-23% had hypogonadism, 1-4% had neurological complications, 4-34% had gastrointestinal complications, 38% had respiratory complications,

52% had urinary system complications, 24% had musculoskeletal system complications, and 2% had osteoporosis.

The physical late effects of treatment on pediatric cancer survivors are the subject of many studies in HICs, which provide critical information. However, the value of HIC information for LMICs is restricted by the significant disparities between the underlying diagnosis, the intensity of the treatment administered, survival rates, and the availability of supportive care treatment options in LMICs and HICs. This compilation reveals the extent of the knowledge gap in this field. However, it has significant limitations, including the complete absence of data from low-income countries, the fact that approximately half of the studies are sourced from India, and the fact that the long-term effects of treatment had not been universally screened for in most studies. As the authors note, there is an urgent need for well-designed large cohort studies with standardized definitions to enhance the quality of life and reduce morbidity among cancer survivors in LMICs.

Reference

1. Turcotte LM, Liu Q, Yasui Y, et al. Temporal Trends in Treatment and Subsequent Neoplasm Risk Among 5-Year Survivors of Childhood Cancer, 1970-2015. *JAMA*. 2017;317(8):814–824. doi: 10.1001/jama.2017.0693

## 13.15. The I-CAH registry: a platform for international collaboration for improving knowledge and clinical care in congenital adrenal hyperplasia

Tseretopoulou X, Bryce J, Chen M, McMillan M, Lucas-Herald AK, Ali SR, Ahmed SF

*Clin Endocrinol (Oxf)*. 2023 Aug 21.
doi: 10.1111/cen.14961. PMID: 37602832.

Brief summary: The authors describe the development and utility of an international collaborative database in congenital adrenal hyperplasia (CAH). I-CAH serves as a tool for benchmarking clinical care and supporting research and development of novel therapies.

Rare disease registries can form the basis of best practice guidelines and allow for monitoring of new drugs and therapeutic interventions. The I-CAH Registry was developed as an international platform to collect standardized data on patients with Congenital Adrenal Hyperplasia (CAH) and was launched in 2014. It is designed to collect comprehensive data across the lifespan of individuals with CAH, allowing for robust research and clinical benchmarking. The registry also supports numerous research projects worldwide.

Up to February 2023, I-CAH included 2690 cases of CAH from 92 centers in 32 countries. Of these, 2410 (90%) were due to 21-hydroxylase deficiency, 90 (3.5%) were due to 11-β hydroxylase deficiency. Other cases included 40 cases of 3β-hydroxylase deficiency (1.5%) and 13 cases of cytochrome P450 oxidoreductase deficiency (0.5%). It includes core demographic data, birth data including sex assignment and details of the condition including genetics. Other data fields include a variety of characteristics at first assessment including prenatal diagnosis, prenatal dexamethasone treatment, presence of adrenal or salt-losing crisis at presentation, current medications and anthropometric data. Longitudinal assessments for bone health, pubertal timing and adverse events are also included. I-CAH has facilitated over 20 research publications and supports ongoing studies that explore various aspects of CAH management and outcomes. It also contributes to care quality improvement (CQI) projects by providing benchmarks and feedback to participating centers.

The I-CAH Registry is an important international resource that enhances the understanding and management of CAH through collaborative research and data-driven quality improvement initiatives. Its robust governance structure and commitment to stakeholder engagement ensure that it remains a valuable tool for advancing CAH care globally.

References

1. Ali S, Lucas?Herald A, Bryce J, Ahmed S. The role of international databases in understanding the aetiology and consequences of differences/disorders of sex development. *Int J Mol Sci.* 2019;20(18): 4405.
2. Kourime M, Bryce J, Jiang J, Nixon R, Rodie M, Ahmed SF. An assessment of the quality of the I-DSD and the I-CAH registries—international registries for rare conditions affecting sex development. *Orphanet J Rare Dis.* 2017;12(1):56.

## 13.16. International newborn screening practices for the early detection of congenital adrenal hyperplasia

Conlon TA, Hawkes CP, Brady JJ, Loeber JG, Murphy N
*Horm Res Paediatr.* 2024;97(2):113-125.
doi: 10.1159/000530754. PMID: 37231960.

Brief summary: Countries differ in their approaches to newborn screening (NBS) for congenital adrenal hyperplasia (CAH), a group of genetic disorders affecting adrenal steroidogenesis. This survey describes the protocols, approaches, and outcomes of CAH screening across different countries.

Members of the International Society for Neonatal Screening were invited to complete a questionnaire in 2021. Members representing 23 screening programs from Europe, Southeast Asia, Australasia, and South America responded to the survey. Of these 21 programs had current active CAH screening and 2 programs were planning to start CAH NBS in the next year. Data on the screening process and outcomes for 2020 were collected.

The study found significant differences in how countries conduct NBS for CAH, in the timing, testing methods, and interpretation of results. Most programs perform sampling between 48 and 72 hours after birth, but other countries allow earlier or later collection. Laboratory techniques vary from a single national laboratory, to multiple labs using standardized protocols, to differing lab protocols within the same country. More than half of programs use a single-tier testing protocol measuring 17OHP by immunoassay. Nine screening programs have introduced or planned to use second-tier testing to reduce false positives. Many programs adjust the 17OHP cutoff values based on gestational age or birth weight to improve screening accuracy. However, the methods for determining these cutoffs are not standardized, leading to further variability. 19 programs provided data on the numbers of– this ranged from 4,500–700,000 infants per year (median 46,000 per year). Participation rates were high $\geq$99% in the programs reporting this data.

This study highlights the challenges in implementing effective CAH screening programs, including the balance between reducing false positives and ensuring early detection. It highlights the large variation in methods used, and indicates potential for standardization across national programs to optimize the early detection of CAH and improve outcomes for affected infants.

References

1. Clayton PE, Miller WL, Oberfield SE, Ritzén EM, Sippell WG, Speiser PW, et al. Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. *Horm Res.* 2002;58(4): 188–95.
2. Pang S, Shook MK. Current status of neonatal screening for congenital adrenal hyperplasia. *Curr Opin Pediatr.* 1997;9(4):419–23

## 13.17. Global health disparities in childhood rickets

Diaz-Thomas A, Iyer P
*Endocrinol Metab Clin North Am*. 2023 Dec;52(4):643-657.
doi: 10.1016/j.ecl.2023.05.011. PMID: 37865479.

Brief summary: This review describes the widespread global issue of childhood rickets, focusing on how disparities in health, nutrition, and environmental factors contribute to its prevalence.

Nutritional rickets is primarily caused by deficiencies in vitamin D or calcium, often due to inadequate nutrition or lack of sunlight exposure. This leads to impaired bone development in children, with potential lifelong consequences. Rickets has historically been more prevalent in marginalized populations, particularly in industrialized cities with high pollution and poor access to nutrition.

Rickets disproportionately affects vulnerable populations, particularly those in low-income countries, immigrant communities in higher-latitude regions, and groups experiencing poor nutrition or limited sunlight due to cultural practices or environmental factors. High rates of rickets are observed in various parts of the world, including Southeast Asia, the Middle East, Africa, and immigrant communities in Europe and North America. Factors such as migration, urbanization, and climate also contribute to these disparities.

The authors emphasize the importance of addressing rickets through global public health policies, such as vitamin D supplementation, dietary fortification, and education about proper nutrition for infants, children, and

pregnant women. Additionally, they discuss the need for more randomized clinical trials to establish the best treatment regimens.

There is the critical need for global policies and investments to address the root causes of rickets, particularly in underprivileged and marginalized populations, to prevent long-term health impacts like osteoporosis in adulthood.

References

1. Kajakumar K, Thomas SB. Reemerging Nutritional Rickets: A Historical Perspective. *Arch Pediatr Adolesc Med.* 2005;159(4):335–341. doi: 10.1001/archpedi.159.4.335a.
2. Munns CF, Shaw N, Kiely M, et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. *J Clin Endocrinol Metab* 2016; 101(2):394–415.

## 13.18. Changing trends in the global, regional, and national burden of iodine deficiency among adolescents and young adults: population-based study

Gong B, Wang C, Yang W, Shan Z

*Eur J Pediatr.* 2024 Jul;183(7):2855-2863.
PMID: 38592486.

Brief summary: This prevalence study examined global, regional and national trends in iodine deficiency among adolescents and young adults, based on data from the Global Burden of Disease (GBD) 2019 database.

Iodine deficiency is a significant public health concern as it can result in hypothyroidism, goiter, and alterations in growth and development. While universal salt iodination has had a significant impact, there is a need for better estimates of the prevalence to guide government strategies and preventive research. The GBD 2019 database was developed by the World Health Organization (WHO) and World Bank and includes information from 204 countries with data on the global burden of 369 diseases and injuries. Countries are divided into six regions including Africa, Eastern Mediterranean, Europe, the Americas, Southeast Asia, and the Western Pacific, and then also divided into five sociodemographic index (SDI) quintiles.

Analysis of global trends reveals that from 1990 to 2019, there was a significant global decline in iodine deficiency prevalence among adolescents. The rates reduced from 3,082 per 100,000 individuals in 1990 to 2,190 per 100,000 in 2019, likely due to implementation of Universal Salt Iodination (USI) in countries with previously high rates of deficiency. The Disability-Adjusted Life Year (DALY) rate for iodine deficiency also decreased globally, indicating overall improvement in the public health impact of iodine deficiency. In 1990, Southeast Asia had the highest prevalence of iodine deficiency, but by 2019, Africa had taken over. Southeast Asia saw the most rapid decline in prevalence rates. The Eastern Mediterranean was the only region where the prevalence of iodine deficiency increased. Analyses by age and sex found that young adults (aged 20–24) accounted for most cases of iodine deficiency in 2019. Both male and female adolescents saw a significant decrease in iodine deficiency rates from 1990 to 2019. Low-SDI countries had the highest prevalence and DALY rates.

Although there has been a substantial global decline in the burden of iodine deficiency among adolescents, it remains a major health concern in low-SDI countries. Evidence from individual countries, including Democratic Republic of Congo, India and China, indicates that implementation of USI has had a significant impact, although this is dependent on the utilization of iodized salt by individual households. The authors recommend continued efforts to strengthen monitoring systems and encourage implementation of effective iodine supplementation measures, particularly in the most affected regions.

References

1. Milevska-Kostova N et al (2022) Universal salt iodization potentially contributes to health equity: socio-economic status of children does not affect iodine status. *J Pediatr Endocrinol Metab* 35(9):1154–1160.
2. GBD 2019 Diseases and Injuries Collaborators (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 396(10258):1204–1222

# 14. The Year in Science and Medicine

Christa E. Flück, Simge Eren, Ken Ong

This year my team in Bern (Philipp Augsburger, Claudia Böttcher, Therina du Toit, Marco Janner, Chrysanthi Kouri, Idoia Martinez de LaPiscina, Rawda Na'Amneh Elzenaty, Anne Smit, Isabel Sousa Barata, Jibira Yakubu) and the YES member, Simge Eren, Şişli Hamidiye Etfal Training and Research Hospital, Pediatric Endocrinology Department, Istanbul participated in choosing the articles you will find in this Chapter.

## Basic Biology and Molecular Mechanisms

### 14.1. The maintenance of oocytes in the mammalian ovary involves extreme protein longevity

Katarina Harasimov, Rebecca L. Gorry, Luisa M. Welp, Sarah Mae Penir, Yehor Horokhovskyi, Shiya Cheng, Katsuyoshi Takaoka, Alexandra Stützer, Ann-Sophie Frombach, Ana Lisa Taylor Tavares, Monika Raabe, Sara Haag, Debojit Saha, Katharina Grewe, Vera Schipper, Silvio O. Rizzoli, Henning Urlaub, Juliane Liepe, Melina Schuh
*Nature Cell Biology*, Volume 26, July 2024: 1124–1138.
doi: 10.1038/s41556-024-01442-7

Brief summary:The female ovary is essential for reproduction. Oocytes are stored in it for more than a year in mice, and for more than four decades in humans. This study investigated proteostasis in oocytes and ovaries of mice by combining quantitative mass spectrometry (MS), pulse–chase labelling, single-cell RNA-seq and nanoscale secondary ion MS (NanoSIMS). They found extraordinary stability of hundreds of proteins. Long-lived proteins were enriched in diverse cellular components (e.g., mitochondria, ribosome, spliceosome, proteasome, chromatin, kinetochore and cytoskeleton) and functions (e.g., metabolism, chaperones, DNA repair and antioxidants). Long-lived somatic cells, including granulosa and stromal cells, as well as individual cells in the theca layer were also identified. The demise of oocytes and the ovary with aging was paralleled by the eventual loss of highly stable proteins.

The ovarian follicle pool plays an important role in female fertility, but insight into its formation, maintenance and decay is missing. Primary or premature ovarian failure as seen with Turner syndrome is a frequent disorder that disables fertility. Basic studies to understand how the ovarian follicle pool is preserved, and what proteins and pathways are involved, are key to understand its normal biology and related diseases. This study provides an enormous amount of data including an atlas of the abundance of ovarian proteins and their changes throughout development. It is fascinating to learn that the ovaries have a $>10$-fold higher fraction of extremely long-lived proteins than other post-mitotic tissues. This guarantees the maintenance and intactness of oocytes over a longer time period. However, with aging even ovarian long-lived proteins are lost. Their identification may help in uncovering the principles involved in resetting the aging clock and inform new therapeutic strategies to delay age-related diseases.

Another interesting article related to the topic informs SF-1 as a key regulator of the formation of the ovarian reserve.

Related literature:

***Exceptional longevity of mammalian ovarian and oocyte macromolecules throughout the reproductive lifespan***

*Ewa K Bomba-Warczak, Karen M Velez, Luhan T Zhou, Christelle Guillermier, Seby Edassery, Matthew L Steinhauser, Jeffrey N Savas, Francesca Elizabeth Duncan bioRxiv [Preprint]. 2024 Jul 5:2023. doi: 10.1101/2023.10.18.562852*

***Steroidogenic factor 1 (SF-1; Nr5a1) regulates the formation of the ovarian reserve.***

*Camilla H. K. Hughes, Olivia E. Smith, Marie-Charlotte Meinsohn, Mylène Brunelle, Nicolas Gévry, Bruce D. Murphy PNAS 2023, Vol. 120 No. 32, e2220849120. doi: 10.1073/pnas.2220849120*

## 14.2. Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation

Young S, Schiffer C, Wagner A, Patz J, Potapenko A, Herrmann L, Nordhoff V, Pock T, Krallmann C, Stallmeyer B, Röpke A, Kierzek M, Biagioni C, Wang T, Haalck L, Deuster D, Hansen JN, Wachten D, Risse B, Behre HM, Schlatt S, Kliesch S, Tüttelmann F, Brenker C, Strünker T.

*J Clin Invest*. 2024 Jan 2;134(1):e173564.
doi: 10.1172/JCI173564

Short summary: This study examined ex vivo the function of the sperm-specific CatSper-channel in almost 2300 men undergoing investigation for infertility. In 9 infertile men with normal semen analysis but pathologic sperm motility, biallelic variants in genes forming the CatSper-channel were found, either in *CATSPER2 or CATSPERE*. These men and their partners failed to conceive naturally and medically assisted reproduction (MAR) was successful only via intracytoplasmic sperm injection (ICSI). The study team developed a 'CatSper-Activity-Test', which was used in combination with [Ca2+]i fluorimetric and electrophysiological recordings to systematically assess the function of CatSper in human sperm, showing the patho-aetiology of CatSper deficiency.

In mammals, sperm motility is controlled by changes in the intracellular Ca2+ concentration through CatSper. Previous studies in mice showed that targeted ablation of genes encoding CatSper subunits did not affect sperm number, morphology, or basal motility, but resulted in failure to switch to the so-called hyperactive motility mode. Although human genetic variants in genes of the CatSper channel were described before, their disease-causing mechanism was unclear.

This study shows that CatSper-deficient human sperm are unable to switch into the hyperactivity mode necessary to penetrate the egg coat for fertilization. This explains the infertility of CatSper-deficient men and the failure of MAR support via ovulation induction, intrauterine insemination, or *in vitro* fertilization, which are currently methods of first choice for couples with unexplained infertility.

After current investigations for infertility in men, one third remains unexplained. It is estimated that variants in genes forming the CatSper channel complex are the most common underlying cause for this group. Thus, the study team suggests maybe rightfully that their "CatSper-Activity-Test" could be performed as part of routine semen analysis to identify patients with a sperm channelopathy and inform personalised selection of the MAR technique. This may reduce time to reproductive success, psychological burden and expenses for many affected couples.

As a final note: So far, genetic analysis of families with clustering of male-factor infertility (due to consanguinity) identified individuals with disease-causing variants in the *CATSPER1-3* genes, the *CATSPERE* gene and with a homozygous deletion of *CATSPER2* and the contiguous gene *STRC*. The latter was termed deafness-infertility syndrome (OMIM 611102) because deletion of *STRC* causes mild-to-moderate hearing loss, which might serve as a diagnostic hint towards defective CatSper when found in infertile men.

## 14.3. The RNA secondary structure of androgen receptor-FL and V7 transcripts reveals novel regulatory region

Warren B. Rouse, Van S. Tompkins, Collin A. O'Leary, Walter N. Moss

*Nucleic Acids Research*, 2024, 52, 6596–6613.
doi: 10.1093/nar/gkae220

Brief summary:This basic research study examined post-transcriptional regulation of the human androgen receptor (AR) using three different human cell lines. The authors studied the structure of two key AR isoforms, full length (AR-FL) and truncated (AR-V7), by computational and experimental analyses and revealed novel insights into the functionally relevant structures in the 5′ and 3′ UTRs of AR-FL for AR expression.

The AR plays important roles for numerous human disorders including androgen insensitivity syndrome (AIS) and various cancers, e.g. prostate cancer. AR has been well studied at the genetic and protein level, but there is still much to be learned about its regulation at the mRNA level to fill gaps in knowledge and find novel targets for treatment of

related disease. Therefore, basic studies are essential. Post-transcriptional regulation of mRNA can be controlled by RNA secondary structure, which may affect splicing, translation, degradation, and localization. These regulatory structures are predominantly localized in the 5′ and 3′ untranslated regions (UTRs) of genes, as studied by Rouse et al for the AR. They can provide access for regulators such as RNA binding proteins and microRNAs.

Understanding the complex (post-transcriptional) regulation of the AR is vital in understanding and treating of related diseases as we still don't know the patho-aetiology of many of these.

Related article showing novel regulatory mechanisms of AR expression involved in AIS:

**Formin-mediated nuclear actin at androgen receptors promotes transcription.**

Reference

1. Julian Knerr, Ralf Werner, Carsten Schwan, Hong Wang, Peter Gebhardt, Helga Grötsch, Almuth Caliebe, Malte Spielmann, Paul-Martin Holterhus, Robert Grosse, Nadine C. Hornig. *Nature* 617, 616–622 (2023). doi: 10.1038/s41586-023-05981-1.

## 14.4. Fetal manipulation of maternal metabolism is a critical function of the imprinted Igf2 gene

Lopez-Tello J, Yong HEJ, Sandovici I, Dowsett GKC, Christoforou ER, Salazar-Petres E, Boyland R, Napso T, Yeo GSH, Lam BYH, Constancia M, Sferruzzi-Perri AN.

*Cell Metab.* 2023 Jul 11;35(7):1195–1208.e6.
doi: 10.1016/j.cmet.2023.06.007

Brief summary:This study in mice shows that insulin-like growth factor 2 (IGF2), encoded by an imprinted gene and expressed by placental endocrine cells, is essential for the adaptive changes in maternal glucose and lipid metabolism during pregnancy to promote fetal growth. Failure of this adaptive program during pregnancy also resulted in metabolic dysfunction of offspring mice later in their life.

IGF2 has been longer known to be involved in the regulation of fetal growth, but its exact mechanism of action was unclear. The imprinted gene Igf2 is highly expressed in (murine) placental cells. This study shows that Igf2 controls placental hormone production (e.g. prolactins) and establishes pregnancy-related insulin resistance to re-allocate nutrients to the fetus. The researchers also show that lack of placental Igf2 causes growth restriction and hypoglycemia in the fetus and also has long-lasting metabolic consequences reaching beyond fetal life. It illustrates how the fetus manipulates the metabolism of the mother to receive enough glucose.

Understanding how fetal factors like IGF2 affect maternal metabolism may help to better comprehend conditions such as gestational diabetes or preeclampsia, potentially leading to improved strategies for managing these pregnancy complications.

This study showing how the fetus and mother interact to regulate the glucose/insulin metabolism between them in pregnancy, was summarized in a 'Research Highlight' in (1) illustrating the importance of this novel mechanistic finding.

Reference

1. Fetal orchestration of maternal metabolism via IGF2. *Nat Rev Endocrinol.* 2023 Oct;19(10):557; doi: 10.1038/s41574-023-00884-7.

## 14.5. Deep learning models reveal replicable, generalizable, and behaviorally relevant sex differences in human functional brain organization

Srikanth Ryalia, Yuan Zhanga, Carlo de los Angelesa, Kaustubh Supekara, Vinod Menona

*PNAS* 2024, Vol. 121, No. 9, e2310012121.
doi: 10.1073/pnas.2310012121

Brief summary:This study used functional MRI to investigate brain organization with a spatiotemporal deep neural network model and explainable AI (XAI) analytics. It found highly significant differences between the

male and female brain on a large number of healthy young probands. It reveals that sex differences in functional brain dynamics are replicable, generalizable and related to sex-specific behavior.

Human brain development is affected by the sex (hormones) throughout life. Sex differences in brain functioning have been found in many disorders, e.g. neurological and psychiatric diseases. While females exhibit more often multiple sclerosis, dementia, anxiety, eating disorders and depression, males are more likely to suffer from Parkinsons, attention-deficit hyperactivity disorder, autism and schizophrenia.

So far, our understanding of brain sex differences has been largely based on anatomical studies and structural brain imaging showing clear sex-specific patterns. However, it was unclear how these structural differences correlate with functional sex differences. Although studies using fMRI seem able to address this question by connectivity analyses, data so far were inconsistent mostly due to methodological issues. To overcome these challenges, this study used data of three (larger) cohorts of healthy, age-'restricted' probands and a refined model to analyze fMRI combined with novel XAI. They claim that there are i) reliable, ii) reproducible, iii) interpretable differences in functional brain organization between typical biological males and females, that iv) clearly relate to sex-specific behavior.

Overall, this study highlights biological sex as an important determinant of human brain organization and challenges the hypothesis that male-female brain organization is a continuum. As this study was performed on young adults aged 20–35 years, it will be very interesting to see similar studies performed on newborns, through childhood and puberty informing on sex development of the human brain. Maybe such studies will also help in understanding sex- and gender-specific behavior and preferences. Further studies could address effects of endogenous and exogenous hormones on brain organization and this might be informative for understanding and managing gender incongruence.

## Epi-/Genetics

### 14.6. Long-read whole-genome analysis of human single cells

Joanna Hård, Jeff E. Mold, Jesper Eisfeldt, Christian Tellgren-Roth, Susana Häggqvist, Ignas Bunikis, Orlando Contreras-Lopez, Chen-Shan Chin, Jessica Nordlund, Carl-Johan Rubin, Lars Feuk, Jakob Michaëlsson, Adam Ameur
*Nature Communications* (2023) 14:5164.
doi: 10.1038/s41467-023-40898-3

Brief summary:Using a new method, long DNA fragments from single human T-cells were sequenced after refined automated single-cell processing and droplet-based whole-genome amplification using PacBio HiFi sequencing. Compared to short-read technologies, the new long-read method markedly improved analyses of genetic variants, especially complex structural variations and variants in repeat elements; it also allowed de novo assembly of parts of the single-cell genome.

Long-read whole-genome analysis is a new genetic method characterized by fragment reads thousands of bases long (compared to 50–300 base pairs with short-reads). This allows for better reconstruction of complex genomic regions, structural variations, as well as repetitive sequences. It therefore provides a more comprehensive view of the genome.

Human single cell whole-genome sequencing is also a new field of interest. It has potential to provide deeper insight into cell biology, including somatic genetic variation, de novo mutation rates, tumor evolution, or meiotic recombination of germ cells. So far technical challenges precluded the use of long-read WGS for single cell analysis, but these now provide novel methodological solutions to overcome these problems.

Is this just about methods? – Clearly no! In the past, all advances in genetic methods resulted in an 'avalanche' of progress in many fields of biology. It is therefore important to get informed about new techniques as they may open doors for solving open research questions that we could not solve because of unsurpassed methodological issues.

## 14.7. Epigenetic inheritance of diet-induced and sperm-borne mitochondrial RNAs

A. Tomar, M. Gomez-Velazquez, R. Gerlini, G. Comas-Armangué, L. Makharadze, T. Kolbe, A. Boersma, M. Dahlhoff, J.P. Burgstaller, M. Lassi, J. Darr, J. Toppari, H. Virtanen, A. Kühnapfel, M. Scholz, K. Landgraf, W. Kiess, M. Vogel, V. Gailus-Durner, H. Fuchs, S. Marschall, M. Hrabě de Angelis, N. Kotaja, A. Körner, R. Teperino

*Nature* 630, 720–727 (2024).
doi: 10.1038/s41586-024-07472-3

Brief summary:This study in mice and humans shows how paternal overweight at conception affects the metabolism of their offspring. Sperm-borne mt-sncRNAs (mitochondrial encoded or derived small non-coding RNAs) are altered by a 2-week high fat diet (HFD) challenge in mice, likely through specific upregulation of mt-tsRNAs (mitochondrial transfer RNAs) caused by mitochondrial dysfunction. In humans, mt-tsRNAs in spermatozoa correlate with body mass index. Genetically engineered mice breeding experiments demonstrate how paternal HFD alterations are passed on to offspring by sperm-to-oocyte transfer of mt-tRNAs at fertilization, and thereby influence the early-embryo transcriptome permanently.

It is well known that parental overweight increases the offspring obesity risk and that obesity has multiple adverse health implications. Although the risk for the offspring is higher when the mother is obese, paternal overweight also doubles this risk.

This extensive study shows in great detail and by sophisticated experiments the mechanism how an environmental factor (here HFD) can alter sperm by non-Mendelian genetic means that can then be passed on to the fetus through sperm-to-oocyte interaction. It shows that environmental factors influencing the parents around the time of conception can affect fetal development fundamentally and permanently by epigenetic inheritance of mt-tRNAs.

This study brings new evidence that ‘we are not only what we eat’ but that offspring metabolism is also influenced by parental eating habits and metabolic health at fertilization.

## 14.8. Histone H2A Lys130 acetylation epigenetically regulates androgen production in prostate cancer

Nguyen T, Sridaran D, Chouhan S, Weimholt C, Wilson A, Luo J, Li T, Koomen J, Fang B, Putluri N, Sreekumar A, Feng FY, Mahajan K, Mahajan NP.

*Nat Commun*. 2023 Jun 9;14(1):3357.
doi: 10.1038/s41467-023-38887-7

Brief summary:This study reveals how castration resistant prostate cancers (CRPC) can produce androgens and become resistant to the inhibitor of androgen production abiraterone. The authors uncover dual phosphorylated-SREBF1 as a sensor of androgen deficiency. Its nuclear translocation and deposition of H2A-K130ac epigenetic marks activates a distinct transcription program that includes SREBF1. This will then lead to intratumoral cholesterol and androgen biosynthesis, liberating CRPCs from their dependence of testicular androgen production and escaping the inhibition due to abiraterone treatment. Thus, SREBF1 Tyr673/951-phosphorylation or the H2A-K130ac epigenetic mark could become new treatment targets.

Prostate cancers (PCa) depend initially on testicular testosterone to flourish. But castration or treatment with the CYP17 inhibitor arbiraterone becomes inefficient within a short time resulting in castration resistant prostate cancers (CRPC). Previously, it was observed that prostate cancers exhibit a distinct shift towards de novo lipid biosynthesis and steroidogenesis. Here, it is now shown that this shift is mediated by the protein SREBF1 and the enzyme GCN5, which modify histones to activate genes responsible for cholesterol and lipid production, and that this leads to enhanced androgen production within the cancer tissue. Researchers also demonstrate in mice that inhibiting these pathways with specific drugs, including afatinib (an EGFR inhibitor) and a GCN5 inhibitor, significantly reduces tumor growth, suggesting new treatment approaches.

This study demonstrates that androgen production in tumors can be enhanced through very complex regulation of lipid biosynthesis and epigenetic modulation that differs fundamentally from normal physiology.

Understanding such alternative (tumor-specific) pathways provides new perspectives on biology and may provide clinical opportunities for diagnostic and therapeutic interventions in other androgen-related disorders, e.g., PCOS.



## 14.9. Sex differences orchestrated by androgens at single-cell resolution

Fei Li, Xudong Xing, Qiqi Jin, Xiang-Ming Wang, Pengfei Dai, Ming Han, Huili Shi, Ze Zhang, Xianlong Shao, Yunyi Peng, Yiqin Zhu, Jiayi Xu, Dan Li, Yu Chen, Wei Wu, Qiao Wang, Chen Yu, Luonan Chen, Fan Bai, Dong Gao
*Nature* 629, 193–200 (2024).
doi: 10.1038/s41586-024-07291-6

Brief summary:This study assembled a single-cell transcriptomic atlas representing over 2.3 million cells from 17 tissues in mice. Investigations of the scRNA-seq data focussed on effects of sex and androgens on the molecular programs and cellular populations in female and male mice as well as male androgen-deprived, and female androgen-treated mice. Data were then used to gain novel molecular insight into sex-related human disorders as available in the UK Biobank study.

Transcript data quantify the active (expressed) genes in a cell, thereby offering insights into cellular function and regulation. Therefore, this new atlas of single-cell transcriptomics data created from many different tissues in mice serves as a rich resource (library) for research questions in mice and beyond. The authors claim that this single-cell atlas may identify cellular targets for sex-biased diseases (e.g. especially those dependent on androgen action) based on the expression patterns of risk genes. They demonstrate this point using human ICD coded disease and genotype data from the UK Biobank study.

The study design - to produce scRNA-seq data, not only in healthy male and female mice, but also in androgen-deprived males and androgen treated-females – enabled novel insights into the role of androgens in sex differences. As sex differences are seen in many and diverse mammalian complex traits, a systemic approach was taken including cells of many different organs. The main finding was that the effect of androgens was positively correlated with sex differences in tissue cell composition. Additionally, known sex differences in the immune system and response were confirmed and an essential role was found for MHC genes in sex differences of human diseases. Some of the observed sex-specific effects could be modulated by targeting the androgen pathway.

Helpfully, the authors developed a web tool to allow customized visualization of their data and a computational pipeline to explore the primary and secondary effects of the androgen–androgen receptor (https://casadbtools.com/andr_effect).

## 14.10. Maternal obesity impacts fetal liver androgen signalling in a sex-specific manner

Ashley S. Meakin, Peter W. Nathanielsz, Cun Li, Vicki L. Clifton, Michael D. Wiese, Janna L. Morrison
*Life Sciences* 337 (2024) 122344.
doi: 10.1016/j.lfs.2023.122344

Short summary: This study describes sex differences in fetal liver-specific androgen signalling that are altered in response to maternal obesity in baboons. It reveals that livers of male fetuses favour a pro-androgenic environment in response to maternal obesity by suppressing the activity of testosterone-metabolising CYP enzymes (CYP2B6 and CYP3A) and by reducing cytoplasmic and nuclear androgen receptor (AR-45) expression. By comparison and most interestingly, there were minimal changes in hepatic androgen signalling in females in response to maternal obesity.

This study adds to the growing evidence that females and males generate distinct adaptations to similar intrauterine environments. Changes to the molecular regulation of hepatic androgen signalling in response to maternal obesity results in a male-specific pro-androgenic environment that may contribute to early programming of liver dysfunction and disease in later life.

Questions remain how exactly maternal obesity causes this sex-specific regulation of enzymes and AR expression in the fetal liver and how this could lead to a permanently altered program of liver function resulting in adult liver disorders. An unsolved conundrum is also that changes in liver function in the male fetus with maternal obesity relate to increased androgens, while in adult men liver diseases relate to decreased androgens; how to explain this switch? Overall, we seem still far from understanding long-term pathways of possible adverse events occurring during pregnancy on later disease of offspring in adult life. Therefore, the authors' idea - that targeting androgen signalling in pregnancies of obese mothers could prevent liver disease in later life of males - is hypothetically correct but far from ready for clinical testing.

## 14.11. Gut bacteria convert glucocorticoids into progestins in the presence of hydrogen gas

Megan D. McCurry, Gabriel D. D'Agostino, Jasmine T. Walsh, Peter J. Turnbaugh, Jun R. Huh, A. Sloan Devlin
*Cell* 2024 Vol. 187 Issue 12 Pages 2952–2968 e13.
doi: 10.1016/j.cell.2024.05.005

Brief summary:This study using mice and human models, shows that human gut bacteria *Gordonibacter pamelaeae* and *Eggerthella lenta* convert biliary corticoids into progestins through 21-dehydroxylation. It thereby shows that a class of immuno- and metabo-regulatory steroids are transformed into a class of sex hormones and neurosteroids. It also illustrates that gut hydrogen gas production is essential and sufficient to support this metabolic pathway, which is more prominent in late pregnancy.

It is known that bacteria living in symbiosis with the human organism ('the microbiome') can interact positively or negatively with various organ functions. It was known that the gut microbiome interacts with host-produced steroids, but the mechanisms and physiological impact of such interactions was largely unknown. Correlations were previously described between gut microbiome, host phenotypes, and levels of sex- and stress-related steroid hormones. These authors now find historical evidence that specific strains of the gut microbiota can convert the glucocorticoids found in human bile into progestins (via 21-dehydroxylation).

This finding is important as it demonstrates that biliary secretion of glucocorticoid may not only be a negligible metabolic road to dispose of steroids (99% of steroids are disposed in urine), but may serve as important substrates for the production of functionally active progestins, including sex steroids and neurosteroids. It also establishes an important role for (bacterial) fermentation and H2 production in modulating steroid metabolism in the gut. Furthermore, it adds evidence that during pregnancy steroid metabolic pathways are regulated differently. Thus, this study opens a novel chapter of the, so far hidden, contribution of the human gut microbiome as an endocrine organ. Bioactive metabolites produced by gut bacteria may modulate hormonal homeostasis and signaling processes in health and disease.

A commentary was published on this study for its relevance in the field of endocrinology.

Reference

1. Progestin production by the gut microbiota. Greenhill, C. *Nat Rev Endocrinol* **20**, 446 (2024). doi: 10.1038/s41574-024-01013-8.

## 14.12. Discriminatory value of steroid hormones on polycystic ovary syndrome and clustering of hyperandrogenism and metabolic factors

Zheng Wang, Martijn Van Faassen, Henk Groen, Astrid E.P. Cantineau, Anne Van Oers, Anna Van der Veen, James M. Hawley, Brian G. Keevil, Ido P. Kema, Annemieke Hoek
*Endocrine Practice* 30 (2024) 348e355.
doi: 10.1016/j.eprac.2024.01.007

Brief summary:This study tested the discriminatory role of 11-oxygenated androgens to diagnose PCOS in women with obesity, and with ($n = 132$) or without ($n = 83$) PCOS. No discriminatory role was found. Instead, insulin resistance, blood pressure, obesity and androgens were the principal components characterizing obese PCOS, while no major characteristic finding defined obese non-PCOS women.

Numerous studies have addressed the mechanism of disease of PCOS and the inter-relationship between PCOS and obesity, as the prevalence of obesity is highly increased in women with PCOS. Similarly, several studies have shown that the steroid metabolome is (specifically) altered in women with PCOS and/or obesity. However, so far no study found a diagnostic serum steroid signature to discriminate women with and without PCOS. Although 11-oxy steroids contribute to the pool of active androgens and are significantly elevated in women with PCOS, this study shows that they cannot serve as PCOS diagnostic markers.

Once more a study demonstrates the immense complexity and heterogeneity of PCOS. Although recent research has provided new insights into the steroid metabolism and beyond of this common disorder, we are still far from understanding it. Broad 'omics' studies on larger cohorts forming subgroups of PCOS with respect to androgen and/or fertility characteristic are ongoing. Preliminary results presented at recent international endocrine meetings are promising. Hopefully, ESPE YB 2025 will report on some important findings.

## 14.13. Influence of state-of-the-art laboratory techniques on the phenotyping of women with polycystic ovary syndrome in the clinical setting

M. Luque-Ramirez, M. Á. Martinez-Garcia, M. Insenser, E. Fernandez-Duran, A. Quintero-Tobar, T. Fiers, J-M. Kaufman, A.M. Garcia-Cano, M. Rosillo Coronado, L. Nattero-Chavez, H.F. Escobar-Morreale

*J Endocrinol Invest*. 2024 Jun 24.
doi: 10.1007/s40618-024-02416-0

Brief summary:This cross-sectional study investigated assay methods for diagnosing PCOS among 359 premenopausal women presenting with functional androgen excess or hyperandrogenemia. Serum androgens and AMH were measured by both immunoassay and LC–MS/MS, and ovarian ultrasound was performed. Steroid immunoassays used in routine practice were unacceptably inaccurate compared to LC-MS/MS, and 'PCOS-positive' serum AMH measurements were often not confirmed by ovarian ultrasound.

Immunoassays for measuring serum androgens have long been shown to be inaccurate. Instead it has long been recommended to use validated chromatographic, mass spectrometric methods for measuring not only androgens but steroids in general. Evidence-based practice guidelines for the management of PCOS recommend the use of validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) assays for diagnosing biochemical hyperandrogenism in patients with PCOS. However, this has not been implemented broadly in clinical routine.

This comparative study of methods quantifies the inaccuracy (20%) of measuring androgens in PCOS women by immunoassays compared to LC-MS/MS. As a consequence, 17% of the patients were reclassified from hyperandrogenic to non-hyperandrogenic PCOS, and in 5% the diagnosis of PCOS could not be confirmed.

Regarding serum AMH measurements in PCOS, the study shows that AMH had high specificity to predict polycystic ovarian morphology (PCOM) found by ultrasound, but sensitivity was very poor resulting in very poor negative prediction of PCOM.

This study nicely illustrates that methods do matter and may frequently lead to wrong diagnostic labels. Established, easy-to-use routine analyses can be outdated and should be replaced as soon as better methods are available, even if newer methods are more costly and/or tedious.

## 14.14. Metabolic rewiring promotes anti-inflammatory effects of glucocorticoids

Jean-Philippe Auger, Max Zimmermann, Maria Faas, Ulrich Stifel, David Chambers, Brenda Krishnacoumar, R. Verena Taudte, Charlotte Grund, Gitta Erdmann, Carina Scholtysek, Stefan Uderhardt, Oumaima Ben Brahim, Mónica Pascual Maté, Cornelia Stoll, Martin Böttcher, Katrin Palumbo-Zerr, Matthew S.J. Mangan, Maria Dzamukova, Markus Kieler,

Melanie Hofmann, Stephan Blüml, Gernot Schabbauer, Dimitrios Mougiakakos, Uwe Sonnewald, Fabian Hartmann, David Simon, Arnd Kleyer, Anika Grüneboom, Susetta Finotto, Eicke Latz, Jörg Hofmann, Georg Schett, Jan Tuckermann, Gerhard Krönke

*Nature* 2024 May;629(8010):184–192.
doi: 10.1038/s41586-024-07282-7

Brief summary:This study uncovers a novel mechanism for the anti-inflammatory effects of glucocorticoids (GCs) that involves reprogramming of mitochondrial metabolism in macrophages through enhanced production of the anti-inflammatory metabolite itaconate and consequent inhibition of the inflammatory response. GCs have very potent anti-inflammatory properties, but the mechanisms are incompletely understood. GCs also have severe side effects when used in high doses for long-term, and therefore novel drugs are needed that ideally have the positive but not the negative properties of GCs.

This study used different approaches (macrophage cultures, ex vivo human biomaterials and inflammatory mice models) to study the exact mechanism of GCs' effect in macrophages. Selected main findings in brief: GC treatment of LPS-activated, inflammatory 'hot' macrophages provoked a global increase in mitochondrial pyruvate consumption by increasing the enzymatic activity of mitochondrial pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) enzymes stimulating the tricarboxylic acid (TCA; Krebs) cycle in mitochondria. This mitochondrial stimulation resulted in increased production of itaconate, a potent anti-inflammatory molecule that interferes with the transcription of pro-inflammatory genes. Production of itaconate depends on the enzyme aconitate decarboxylase (ACOD1) and its gene expression was significantly altered in LPS-induced macrophages.

In line with findings in macrophages, GCs had a reduced effect on inflammatory cytokines in *Acod1* −/− mice, but the effect could be rescued by itaconate mimetics. Higher itaconate serum levels were found in GC treated patients with rheumatoid arthritis.

Taken together, the anti-inflammatory effects of GCs in different immune-mediated inflammatory diseases (e.g., rheumatoid arthritis or asthma) depend on GC-induced mitochondrial reprogramming. This understanding might provide targets for novel drugs to deliver the anti-inflammatory effects of GCs without their side effects.

## Bone

## 14.15. Dominant negative variants in KIF5B cause osteogenesis imperfecta via down regulation of mTOR signaling

Ronit Marom, Bo Zhang, Megan E. Washington, I-Wen Song, Lindsay C. Burrage, Vittoria C. Rossi, Ava S. Berrier, Anika Lindsey, Jacob Lesinski, Michael L. Nonet, Jian Chen, Dustin Baldridge, Gary A. Silverman, V. Reid Sutton, Jill A. Rosenfeld, Alyssa A. Tran, M. John Hicks, David R. Murdock, Hongzheng Dai, MaryAnn Weis, Shalini N. Jhangiani, Donna M. Muzny, Richard A. Gibbs, Richard Caswell, Carrie Pottinger, Deirdre Cilliers, Karen Stals, Undiagnosed Diseases Network, David Eyre, Deborah Krakow, Tim Schedl, Stephen C. Pak, Brendan H. Lee

*PLoS Genet*. 2023 Nov 7;19(11):e1011005.
doi: 10.1371/journal.pgen.1011005

Brief summary:Heterozygous, de novo variants in *KIF5B* are identified in 4 individuals with osteogenesis imperfecta. Studies of these *KIF5B* variants in *C. elegans* and cell models reveal the disease-causing mechanism. KIF5B seems important for intracellular trafficking and mTOR signaling to maintain skeletal homeostasis.

Several years after the description of the last new osteogenesis imperfecta (OI)-related gene, the authors report on new dominant negative *KIF5B* gene variants in unrelated individuals with OI. KIF5B belongs to the kinesin superfamily proteins that play a key role in cellular function like cell cycle, endocytosis and ciliogenesis. Kinesin-1 heavy chains are encoded by *KIFB* genes. Pathogenic variants in KIF genes lead to multisystemic disorders known as 'kinesinopathies'. They have been associated with variable human phenotypes, including neurodevelopmental disorders and skeletal dysplasias. Previously, *KIFB5* variants were reported in individuals with and without skeletal dysplasias.

The authors describe novel variants of the *KIFB5* gene located in the specific kinesin motor domain of the protein; all manifesting with OI, a new phenotype for kinesinopathies.

Mechanistic studies in bone tissue and fibroblasts of these KIFB5 variant patients revealed a downstream inhibition on mTOR signaling pathway likely responsible for the bone phenotype. By showing positive rescue through reestablishing mTOR signaling with the essential amino acid leucine (a known stimulator of mTOR), the authors confirm the critical role of mTOR in KIFB5 related OI and propose possible targets for future therapeutic approaches.

## 14.16. New horizons: translational aspects of osteomorphs

Kyung-Hyun Park-Min, Se Hwan Mun, Richard Bockman, Michelle M. McDonald
*The Journal of Clinical Endocrinology & Metabolism*, 2024, **109**, e1373–e1378.
doi: 10.1210/clinem/dgad711

Brief summary:This review summarizes findings in mice on a new lineage of osteoclast-related cells that play a role in bone homeostasis at the resorption front. These 'osteomorphs' derive from fission of osteoclasts and are long-lived. They seem to interact with RANKL and may be involved in the rebound bone loss that is seen following denosumab (RANKL antagonist) therapy. The authors speculate on translational roles for osteomorphs in humans, including bone catabolic disorders such as osteoporosis, bone-targeted cancers and chronic inflammation.

Osteoclasts (OCL) are highly specialized multinucleated myeloid cells. OCL differentiate from myeloid stem cells to mononuclear OCL precursors which eventually fuse to multinucleated cells that resorb bone. After a life cycle of 2–3 weeks they were so far believed to undergo apoptosis. The life-cycle of OCL is regulated by an array of cytokines and hormones including PTH, RANKL, osteoprotegerin (OPG) and estrogens.

Several studies in mice recently described a new OCL-related cell type, 'osteomorphs'. These result from fission of mature OCL and can subsequently again undergo fusion to generate multinucleated OCLs, in a novel cell re-cycling process. These studies provide mechanistic insight into the denosumab-withdrawal-rebound phenomenon as well as into other bone metabolic aspects informing about possible targets for interventions.

This review is written from a translational perspective. It summarises preclinical knowledge on osteomorphs in mice and translates this into possible implications in humans. However, osteomorphs have thus far been identified only in mice. Corresponding studies in humans are still lacking.

**YES contributions by Dr. Simge Eren (14.17 and 14.18)**

## 14.17. A maternal brain hormone that builds bone

Muriel E. Babey, William C. Krause, Kun Chen, Candice B. Herber, Zsofia Torok, Joni Nikkanen, Ruben Rodriguez, Xiao Zhang, Fernanda Castro-Navarro, Yuting Wang, Erika E. Wheeler, Saul Villeda, J. Kent Leach, Nancy E. Lane, Erica L. Scheller, Charles K. F. Chan, Thomas H. Ambrosi, Holly A. Ingraham
*Nature* | Vol 632 | 8 August 2024.
doi: 10.1038/s41586-024-07634-3

Brief summary:This study shows that brain-derived cellular communication network factor 3 (CCN3) is a potent osteoanabolic hormone to enhance bone formation in lactating female mice. CCN3 is produced by KISS1 neurons in the brain, and operates through a unique signaling pathway that interacts with bone-forming osteoblasts leading to increased bone formation.

During lactation, the increased calcium demand for milk production leads to considerable bone loss. Normally, estrogen helps counterbalance excessive bone resorption by encouraging bone formation, but levels drop

significantly during the postpartum period. This study shows that CCN3, secreted by KISS1 neurons in the arcuate nucleus (ARCKISS1), can compensate for this estrogen drop and acts as a powerful factor for building bone in lactating females. The study reveals CCN3 as the humoral factor previously suggested to generate the dense bone phenotype in females that enhances bone mass and affects skeletal stem cells to boost their frequency and potential for bone formation (1). CCN3 stimulates skeletal stem cell activity in both mice and humans, enhances bone remodeling, and speeds up fracture repair in mice of all ages and both sexes. The role of CCN3 in female physiology became evident when a surge in CCN3 expression in ARC KISS1 neurons was detected during lactation. Reducing CCN3 levels in these neurons led to bone loss in lactating mothers and impaired their ability to sustain their offspring when exposed to a low-calcium diet.

Maintaining bone health is essential throughout life, with early development being critical for establishing strong bones. The impact of CCN3 on bone growth is particularly notable during developmental stages, affecting bone density and the overall skeletal structure. While previous research has underscored the importance of hormonal regulation in bone health, the discovery of CCN3 introduces a novel mechanism through which brain-derived signals influence bone formation. It suggests that maternal factors have a direct impact on bone development. Imbalances in CCN3 levels might be linked to bone density disorders like osteoporosis. Thus gaining a deeper understanding of how CCN3 functions could pave the way for new treatment approaches.

Related literature:

***1) Estrogen signaling in arcuate Kiss1 neurons suppresses a sex-dependent female circuit promoting dense strong bones.***

Reference

1. Candice B Herber, William C Krause, Liping Wang, James R Bayrer, Alfred Li, Matthew Schmitz, Aaron Fields, Breanna Ford, Zhi Zhang, Michelle S Reid, Daniel K Nomura, Robert A Nissenson, Stephanie M Correa, Holly A Ingraham *Nat. Commun.*10, 163 (2019). doi: 10.1038/s41467-018-08046-4.

## 14.18. EPAC1 enhances brown fat growth and beige adipogenesis

Laia Reverte-Salisa, Sana Siddig, Staffan Hildebrand, Xi Yao, Jelena Zurkovic, Michelle Y. Jaeckstein, Joerg Heeren, Frank Lezoualc'h, Natalie Krahmer, Alexander Pfeifer

*Nature Cell Biology*, Volume 26, January 2024: 113–123.
doi: 10.1038/s41556-023-01311-9

Brief summary:This basic science study, using mice, cell models, and organoids, shows that the cAMP-binding protein EPAC1 is a central regulator of adaptive brown adipose tissue (BAT). EPAC1 specifically increases the number of thermogenic fat cells (brown and beige adipocytes), by promoting the differentiation and growth of brown adipocytes and the development of beige adipocytes through signaling pathways that enhance lipid metabolism and thermogenesis.

BAT plays a crucial role in promoting energy expenditure and enhancing cardiometabolic health by dissipating energy, primarily through a process known as non-shivering thermogenesis, which is facilitated by the uncoupling protein 1 (UCP1). An increase in BAT mass is positively correlated with leanness and a reduced risk of cardiovascular disease in adults. Besides brown fat cells, another type of thermogenic fat cell exists, known as beige cells; they are predominantly located in subcutaneous white adipose tissue (WAT). Similar to brown adipocytes, these beige cells can be induced through exposure to cold or pharmacological interventions, a process referred to as browning or beiging.

The study highlights exchange protein directly activated by cAMP isoform 1 (EPAC1) as a key regulator of adaptive BAT and beige adipogenesis, demonstrating that its pharmacological activation not only increases adipocyte cell mass but also promotes the browning of white fat, resulting in enhanced energy expenditure and reduced diet-induced obesity. EPAC1 specifically drives the proliferation of thermogenic fat cells without impacting white fat cells, and its absence in preadipocytes impairs BAT and beige adipogenesis, exacerbating obesity. Furthermore, activation of EPAC1 (encoded by the gene *RAPGEF3*) supports the proliferation and differentiation of human brown fat cells and organoids, while a coding variant of RAPGEF3, associated with body mass index, hinders norepinephrine-induced proliferation of brown fat cells.

Taken together, these findings suggest that EPAC1 could be a promising therapeutic target for treating obesity and related metabolic disorders by boosting the body's natural fat-burning capabilities. The study also emphasizes the need for a deeper understanding of the molecular mechanisms governing BAT and beige adipogenesis, which could pave the way for innovative strategies to improve metabolic health.

# 15. Editors' Choice

Ken K Ong, Christa E. Flück

## New Treatments

### 15.1. Semaglutide in early type 1 diabetes

Dandona P, Chaudhuri A, Ghanim H.
*N Engl J Med* **389**(10): 958–959 (2023).
PubMed: 37672701

In Brief: This research letter describes a recent case series of 10 patients aged 21–39 years who started taking Semaglutide within 3 months of their diagnosis of antibody-positive type 1 diabetes (T1D), in addition to standard basal and prandial insulin. All 10 patients stopped prandial insulin within 3 months of Semaglutide, and 7 patients also stopped basal insulin by 6 months. Mean HbA1c fell from 11.7% at diagnosis to 5.9% at 6 months and 5.7% at 12 months. Mean fasting C-peptide level increased in all patients from 0.65 to 1.05 ng/ml.

Although this is an uncontrolled case series, the reductions (and stopping) in insulin therapy are striking and the authors report that the reductions in HbA1c are greater than those in the placebo control arms of trials in similar patients. Data are restricted to the first 12 months after T1D diagnosis and therefore may be largely influenced by the honeymoon period. However, 4/10 patients initially presented with diabetic ketoacidosis and the high HbA1c levels at diagnosis suggests this sample is typical of young adults with T1D.

Other recent large case series have described benefits of Semaglutide in patients who have both T1D and overweight or obesity, largely through its established effects on weight loss (1). The current paper suggests that Semaglutide may have more direct benefits on beta cell function – although baseline and changes in weight and BMI were not included in this brief report.

Reference

1. Garg SK. *et al*. Efficacy of Semaglutide in Overweight and Obese Patients with Type 1 Diabetes. *Diabetes Technol Ther*. 2024 Mar;26(3):184–189. doi: 10.1089/dia.2023.0490. PMID: 38444317

### 15.2. A phase 3, randomized, controlled trial of resmetirom in NASH with liver fibrosis

Harrison SA, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, Labriola D, Moussa SE, Neff GW, Rinella ME, Anstee QM, Abdelmalek MF, Younossi Z, Baum SJ, Francque S, Charlton MR, Newsome PN, Lanthier N, Schiefke I, Mangia A, Pericas JM, Patil R, Sanyal AJ, Noureddin M, Bansal MB, Alkhouri N, Castera L, Rudraraju M, Ratziu V, the MAESTRO-NASH Investigators.
*N Engl J Med* **390**(6): 497–509 (2024).
PubMed: 38324483

In Brief This phase 3 trial including 966 adults with biopsy-confirmed non-alcoholic steatohepatitis (NASH) with fibrosis were randomly assigned (1:1:1 ratio) to once-daily resmetirom 80 mg or 100 mg or placebo. Resmetirom, both 80 and 100 mg daily, were superior to placebo with respect to NASH resolution and improvement in liver fibrosis.

NASH resolution occurred more frequently in patients on 80-mg (25.9%) and 100-mg resmetirom (29.9%) than on placebo (9.7%) ($P < 0.001$ for both). The main side-effects of resmetirom were (generally self-limiting) diarrhea and nausea at treatment initiation.

Despite being one of the most common comorbidities of obesity, there are no licenced effective treatments for NASH - although resolution of NASH and associated fibrosis does occur with successful lifestyle weight management. Due to the absence of effective treatments, the US Food and Drug Ad-ministration (FDA) has agreed an accelerated approval pathway for Resmetirom, which is an oral, liver-directed, thyroid hormone receptor (THR) beta-selective agonist.

Previous research showed impaired THR-beta function in the liver in NASH, leading to reduced mito-chondrial function and fatty acid beta-oxidation. This mechanism also explains why some patients with severe untreated hypothyroidism have markedly raised liver enzyme levels and steatohepatitis, which promptly resolves following appropriate thyroid hormone replacement therapy. Resmetirom appears to be a very promising new treatment for NASH with liver fibrosis.

Of note, the Yearbook editors welcome the new name change for NASH to now '*metabolic dysfunction–associated steatohepatitis*' (MASH) – previously, having to explain to parents and children that they had a 'non-alcoholic' condition was always puzzling!

## 15.3. Triple-hormone-receptor agonist retatrutide for obesity – a phase 2 trial

Jastreboff AM, Kaplan LM, Frias JP, Wu Q, Du Y, Gurbuz S, Coskun T, Haupt A, Milicevic Z, Hartman ML, for the Retatrutide Phase 2 Obesity Trial Investigators.
*N Engl J Med* **389**(6): 514–526 (2023).
PubMed: 37366315

In Brief: This phase 2 trial randomized 338 adults with overweight or obesity to once-weekly subcutaneous Retatrutide (LY3437943), a 'triple agonist' for glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP1), and glucagon receptors, or placebo. Mean % change in body weight at 24 weeks was −7.2%, −12.9%, −17.3% and −17.5% in the 1-mg, 4-mg, 8-mg and 12-mg Retatrutide groups, compared with −1.6% in the placebo group.

GLP1 analogues have remarkably good effectiveness in the treatment of Type 2 diabetes and other comorbidities of obesity. Indeed, many of us are hoping for wider funding approvals to enable such therapies to reach more of our Paediatric patients. Meanwhile, drug companies are developing even more potent treatments by combining additional incretin receptor targets, such as the dual-agonist Tirzepatide (GIP and GLP1) and Retatrutide (GIP, GLP1 and Glucagon).

The results are impressive. After 48 weeks treatment, 75%, 91%, and 93% of adults had lost 10% or more body weight on 1-mg, 4-mg, and 8-mg Retatrutide, and 60%, 75% and 83% had lost 15% or more body weight. Larger % reductions in body weight were seen in women, and in those with higher baseline BMI. Similar to other incretin receptor agonists, gastrointestinal side effects were only of mild to moderate severity, were dose-related, and partially mitigated by starting on a low dose.

There will no doubt be many more future incretin-based drugs. Even if they don't combine even more receptor targets, there may be differing combinations of receptor potency. Compared to the endogenous (natural) ligands, Retatrutide shows much higher potency for the GIP receptor (8.9-fold higher), and only half the natural potency for GLP1 and Glucagon receptors. The optimal combination of receptor potencies may be different to this, or might even vary between individuals, e.g. depending on sex, age, health, physical or genetic characteristics.

## 15.4. Anti-insulin receptor antibody for malignant insulinoma and refractory hypoglycemia

Osataphan S, Vamvini M, Rosen ED, Pei L, Erlikh N, Singh G, Dhorajiya P, Parker JA, Dreyfuss JM, Rattani A, Patti ME.
*N Engl J Med* **389**(8): 767-769 (2023).
PubMed: 37611129

In Brief: This case report describes the successful use of RZ358, a novel human anti–insulin receptor monoclonal antibody therapy, in a patient with severe, refractory hypoglycaemia due to metastatic insulinoma.

The tumour and resulting severe hypoglycaemia had been resistant to treatments with octreotide, lutetium Lu 177 dotatate radionuclide, diazoxide, everolimus, dexamethasone, pasireotide, and glucagon – and the patient, a 55-year old man with a pathogenic *MEN1* gene mutation, was dependent on continuous high dose 50% dextrose infusion. So the authors, from the Beth Israel Deaconess Medical Center, Boston, obtained emergency use authorization from the US Food and Drug Administration to try RZ358 for the first time. Hypoglycaemia resolved after the second infusion of RZ358 (6 mg/kg) and all other therapies could be discontinued after an additional 4 infusions. Despite persisting high tumour size, severe hypoglycaemia remained controlled by ongoing monthly infusions (up to 9 months at the time of report).

As well as providing a future effective option for the treatment of resistant hyperinsulinism, this case report highlights the potential for therapeutic monoclonal antibodies (MAB) in metabolic conditions. In recent years, MABs have emerged as remarkably effective new treatments in oncology, and as immunotherapies for rheumatology and other inflammatory diseases. So far, endocrinologists have been mostly involved in 'picking up the pieces' of managing endocrine immune-related adverse events, e.g. thyroiditis (in up to 9%), hypophysitis ($<1\%$) and more rarely primary adrenal insufficiency.

In Paediatric Endocrinology, we use denosumab, a MAB which inhibits osteoclast formation, function, and survival, as a treatment for rare (RANKL)-mediated disorders, and also emerging MAB immunotherapies to prevent or prolong beta-cell function in Type 1 diabetes. Other promising new endocrine targets include MABs that bind to and degrade stimulating thyrotropin receptor autoantibodies as new treatments for Graves' Disease (1).

Reference

1. Wolf J *et al*. A Novel Monoclonal Antibody Degrades the Thyrotropin Receptor Autoantibodies in Graves' Disease. *Endocrine Practice*. Vol 29, 7; 553-559 (2023) 10.1016/j.eprac.2023.04.002

## New Concerns

### 15.5. Iatrogenic Alzheimer's disease in recipients of cadaveric pituitary-derived growth hormone

Banerjee G, Farmer SF, Hyare H, Jaunmuktane Z, Mead S, Ryan NS, Schott JM, Werring DJ, Rudge P, Collinge J.
*Nat Med* **30**(2): 394–402 (2024).
PubMed: 38287166

In Brief: These authors investigated patients referred to the UK National Prion Clinic (NPC) for suspected prion diseases. They identified 8 individuals with a history of receiving treatment with cadaveric pituitary-derived growth hormone (c-hGH) and referred to, or reviewed by, the NPC between 2017 and 2022. None had a diagnosis of iatrogenic Creutzfeldt–Jakob disease (CJD) (on the basis of clinical presentation, neuroimaging and biomarkers and, in two cases, by postmortem examination). Yet, 5 patients fulfilled the diagnostic criteria for Alzheimer's disease (AD).

At least 1,848 patients in the UK were treated with c-hGH between 1959 and 1985. Of these, 80 cases of iatrogenic CJD were recorded, and worldwide there have been over 200 such cases. However, c-hGH resulted in the transmission not only of the CJD prion, but also of amyloid-beta (Abeta) and tau, which are characteristic features of Alzheimer's disease (AD). On histology, these proteins are typically deposited in the brain parenchyma and blood vessels and in neurofibrillary tangles of hyperphosphorylated tau.

This study shows that tragically, the original c-hGH recipients who did not die from iatrogenic CJD may develop iatrogenic AD. The 5 patients with iatrogenic AD had progressive cognitive impairment in two or more domains severe enough to affect usual activities of daily living. Symptoms started between ages 38 and 55 years old and in some cases progression was rapid.

The study also provides compelling insights into the pathogenesis of AD, that Abeta and tau are indeed causal factors for AD, and also that AD is a transmissible disease due to human-human transmission of Abeta and tau proteins. Fortunately, there is no evidence for human-human transmission of Abeta in normal human interactions. However, it underlines the need to prevent iatrogenic transmission of AD (as is already recognised for CJD) during medical and surgical procedures. Furthermore, as AD is far more prevalent than CJD, the likelihood and public health impact of iatrogenic AD is likely to be far greater than that of iatrogenic CJD.

## 15.6. Consumption of 100% fruit juice and body weight in children and adults: a systematic review and meta-analysis

Nguyen M, Jarvis SE, Chiavaroli L, Mejia SB, Zurbau A, Khan TA, Tobias DK, Willett WC, Hu FB, Hanley AJ, Birken CS, Sievenpiper JL, Malik VS.
*JAMA Pediatr* **178**(3): 237–246 (2024).
PubMed: 38227336

In Brief This systematic review collated evidence from prospective cohort studies (PCS) with at least 6 months follow-up and randomized clinical trials (RCTs) to assess the influence of 100% fruit juice on body weight in children and adults. 100% fruit juice intake was associated with higher BMI gain among children and with higher weight gain in adults.

In children, 17 PCS (total 45,851 children) and no RCTs were identified. Each additional serving per day of 100% fruit juice was associated with a 0.03 (95% CI, 0.01–0.05) higher BMI change.

In adults, there were 6 PCS and 19 RCTs (total 268 095). In PCS, each additional serving per day of 100% fruit juice was associated with a 0.21 kg (95% CI, 0.15–0.27) greater body weight gain. The association was significant only among analyses unadjusted for total energy, suggesting that the effect of fruit juice is explained by higher calorie intake. There was no effect on body weight in RCTs, but confidence intervals were wide (mean difference: −0.53 kg; 95% CI, −1.55 to 0.48 kg).

100% fruit juice is widely perceived by the public and even many health professionals as being a 'healthy food'. Indeed it can count towards one of your recommended '5-a-day' portions of fruit and vegetables. However, its content is high in free (simple) sugars – and other types of sweet drinks are robustly associated with higher weight gain and higher risk of Type 2 diabetes in both children and adults. Hence it has been debated whether there is anything especially healthy about the natural sugar (mostly fructose) released directly from fruit. For comparison, most added (or 'table') sugar is sucrose that is extracted from sugar cane or sugar beet, and then refined.

These findings support guidance to limit intakes of fruit juice. While the overall effect sizes on weight and BMI were small, they could add up in individuals with high fruit juice intakes, and contribute significantly to the burden of excess calories at the population level - and sweetened fruit juices are probably even worse. In addition to weight gain, 100% fruit juice may also have adverse effects on blood glucose levels, risk of dental carries, and may lower dietary fibre intake by replacing consumption of whole fruits.

## 15.7. Excess mortality in england post COVID-19 pandemic: implications for secondary prevention

Pearson-Stuttard J, Caul S, McDonald S, Whamond E, Newton JN.
*Lancet Reg Health Eur* **36**: 100802 (2024).
PubMed: 38188277

In Brief: The authors highlight recent data published by the UK Office for Health Improvement and Disparities (OHID) on estimated excess mortality in England, overall and by age, ethnicity, region and cause. Age-standardised mortality was higher than expected during June 2022 to 30th June 2023. Particular causes of death

showed excess mortality: cardiovascular diseases (12% relative excess), heart failure (20%), ischaemic heart diseases (15%), liver diseases (19%), acute respiratory infections (14%), and diabetes (13%).

During the acute phase of the COVID-19 pandemic, the greatest numbers of excess deaths occurred among the elderly. By contrast, since the pandemic there is strong evidence for persisting excess deaths most apparent among middle-aged and younger adults, and particularly due to cardiometabolic diseases. The highest relative excess deaths post-pandemic occurred among middle-aged adults (50–64 years): cardiovascular diseases (33% relative excess), ischaemic heart diseases (44%), cerebrovascular diseases (40%); heart failure (39%), acute respiratory infections (43%), diabetes (35%), liver diseases (19%).

The COVID-19 pandemic prompted enhanced monitoring and many reports have documented substantial adverse changes in physical and mental health, physical activity, and diet, largely attributed to the lifestyle restrictions during periods of population 'lockdown'. We know that rates of overweight and obesity increased rapidly in all age groups, and this is thought to be the main reason for the 2- to 3-fold higher incidence of central precocious puberty, commented on in Yearbook 2023 (https://www.espeyearbook.org/ey/0020/ey0020.5-7) and since systematically reviewed (1).

The current data serve as a warning that enhanced levels of monitoring need to continue. There are few data on the post-pandemic numbers of overweight and obesity (2) - and none on central precocious puberty (has this returned to pre-pandemic rates?). Furthermore, studies are needed to understand whether persisting excess deaths may be due to persisting unhealthy changes in lifestyle behaviours, persisting physical and mental disease, or even some form of metabolic programming.

References

1. Nguyen NN, *et al*. Difference in Precocious Puberty Between Pre-COVID-19 and COVID-19 Periods: A Meta-Analysis. *American Journal of Epidemiology*, 2024 Aug 21:kwae295, Online ahead of print. https://doi.org/10.1093/aje/kwae295
2. Maessen SE, *et al*. High but decreasing prevalence of overweight in preschool children: encouragement for further action. *BMJ*. 2023 Oct 9:383:e075736. doi: 10.1136/bmj-2023-075736.

## 15.8. Causes and consequences of child growth faltering in low-resource settings

Mertens A, Benjamin-Chung J, Colford JM, Jr., Coyle J, van der Laan MJ, Hubbard AE, Rosete S, Malenica I, Hejazi N, Sofrygin O, Cai W, Li H, Nguyen A, Pokpongkiat NN, Djajadi S, Seth A, Jung E, Chung EO, Jilek W, Subramoney V, Hafen R, Haggstrom J, Norman T, Brown KH, Christian P, Arnold BF, The Ki Child Growth Consortium.

*Nature* **621**(7979): 568–576 (2023).
PubMed: 37704722

In Brief: The authors perform a population intervention effects analysis of 33 longitudinal cohorts (total 83 671 children, 662 763 measurements), from 15 low- and middle-income counties (LMICs). They estimate that improving maternal anthropometry and child condition at birth would increase population mean length-for-age *z*-scores by up to 0.40 and weight-for-length *z*-scores by up to 0.15 by 24 months of age.

This is 1 of 3 full articles published in *Nature* in September 2023. The other 2 articles documented the high incidence of childhood wasting and stunting (the highest incidence of both were by age 3 months) and their adverse consequences (persistent growth faltering and child mortality). The studies are funded by the Bill & Melinda Gates Foundation's Knowledge Integration (ki) initiative, which aims to promote growth and development during the first 1,000 days of life, from conception.

Such prominent attention to this topic is welcome. Despite much research and international efforts, interventions to prevent stunting and wasting have only modest effects. The authors conjecture this is due to incomplete understanding of the optimal manner and timing of interventions. For example, there was surprisingly little estimated benefit of exclusive or predominant breastfeeding, or of reducing diarrhoea during the first 24 months.

Instead, after considering 30 separate exposures, the key findings highlight the importance of improving prenatal exposures – taller maternal height and larger child birth size. This will require long-term investments to support broad improvements in living standards, and throughout the mother's childhood. Accordingly, the countries that have shown the greatest reductions in stunting have undergone improvements in maternal education, nutrition and maternal and newborn healthcare and reductions in the number of pregnancies.

## 15.9. Computer-aided facial analysis as a tool to identify patients with Silver-Russell syndrome and Prader-Willi syndrome

Ciancia S, Goedegebuure WJ, Grootjen LN, Hokken-Koelega ACS, Kerkhof GF, van der Kaay DCM.
*Eur J Pediatr* **182**(6): 2607–2614 (2023).
PubMed: 36947243

In Brief: The authors tested the diagnostic ability of the Face2Gene app in 23 children with a clinical or genetically confirmed diagnosis of Silver-Russell syndrome (SRS) and 29 children with genetically confirmed Prader-Willi syndrome (PWS). When combined with careful routine clinical history and examination, the Face2Gene app can be a useful diagnostic tool.

Among PWS patients, Face2Gene calculated the top 1, top 5, and top 10 sensitivities to be 76%, 97%, and 100%, respectively. 'Top 1/5/10' means that the correct syndrome is listed among the top 1/5/10 possible syndromes. Face2Gene app performance was slightly better in PWS patients with paternal deletion of chromosome 15q11-13 than those with maternal uniparental disomy, but showed no difference with age.

Detection of SRS was overall lower: top 1, top 5, and top 10 sensitivities were 39%, 65%, and 91%, respectively – but Face2Gene performed better in younger SRS patients, or if a photo of the child taken at a younger age was available.

Clinical geneticists (and many of our esteemed colleagues) are particularly good at spotting rare genetic disorders based on a patient's facial and other physical characteristics. But genetic testing is becoming more widely available - without needing referral to clinical genetics clinics. This raises the risks that appropriate genetic testing may be omitted or that expensive tests are too frequently requested. Artificial intelligence is extremely good at pattern recognition. The Face2Gene app takes a single frontal photo and uses face detection technology built on deep convolutional neural networks. Specific facial landmarks are extracted and compared to the app's database of information on $>10\,000$ syndromes.

There will be concerns about safety and confidentiality. Face2Gene seems to be secure – original photos uploaded to it are encrypted and stored securely, available only to the individual clinician or researcher who submitted the case - although this will need to be tested and validated by regulators. However, these are striking new findings and likely represent a taste of the many benefits to come for clinical practice in the world of artificial intelligence.

## 15.10. A foundation model for generalizable disease detection from retinal images

Zhou Y, Chia MA, Wagner SK, Ayhan MS, Williamson DJ, Struyven RR, Liu T, Xu M, Lozano MG, Woodward-Court P, Kihara Y, Eye UKB, Vision C, Altmann A, Lee AY, Topol EJ, Denniston AK, Alexander DC, Keane PA.
*Nature* **622**(7981): 156–163 (2023).
PubMed: 37704728

In Brief: The authors present 'RETFound', a self-supervised learning approach that has so far analysed 1.6 million retinal images to enable disease detection. RETFound shows good accuracy for diagnosis and prognosis of sight-threatening eye diseases. It also contributes to incident prediction of complex systemic disorders such as heart failure and myocardial infarction.

The well-known saying 'the eyes are the window to the soul' means that you can guess a person's emotions, thoughts, or inner self by looking into their eyes. This saying is hereby extended to 'the eyes are a window to your complex disease risks'! 'Oculomics' is the concept that retinal images illustrate physical changes associated with (non-ophthalmological) systemic diseases. For example, changes in the optic nerve and inner retinal may share common determinants to central nervous system pathogenesis and neurodegeneration, and changes in retinal vascular geometry may be shared with vascular disorders of the heart and kidneys.

It is impressive enough that RETFound showed good ability to diagnose and classify eye diseases, such as diabetic retinopathy (AUROC =0.943, 0.822 and 0.884 on 3 separate datasets). More remarkably, it also showed good prediction for future diagnosis of ischaemic stroke, myocardial infarction, heart failure and Parkinson's disease. Its self-supervised learning basis means that RETFound can continue to develop itself with additional input data. These academic researchers hope that this type of 'medical foundation model' will democratize access to medical artificial intelligence and accelerate progress towards its widespread clinical implementation.

## 15.11. Google AI has better bedside manner than human doctors – and makes better diagnoses

Mariana Lenharo

*Nature* | Vol 625 | 25 January 2024 | 643-4.

https://www.nature.com/articles/d41586-024-00099-4

In Brief: This 'News in focus' article in the journal *Nature* describes a non peer-reviewed preprint (1) by researchers at Google Research and Google Deepmind that claims remarkable abilities for medical artificial intelligence (AI), beyond 'simple' pattern recognition tasks.

The preprint describes Google's 'Articulate Medical Intelligence Explorer' (AMIE), an experimental conversational AI system, or 'chatbot'. It was tested on 20 trained actors who simulated 149 clinical scenarios. They were blinded to interact with AMIE or one of 20 board-certified physicians. AMIE matched or surpassed physicians' diagnostic accuracy in all 6 medical specialties considered (cardiology, gastroenterology, neurology, obstetrics, respiratory and internal medicine). More notably, AMIE outperformed physicians in 24 of 26 criteria for conversation, including politeness, explanation, and in expressing honesty, care and commitment.

To enable efficient self-learning, AMIE was taught to play 3 different roles: a patient with a specific condition; an empathetic clinician; and a critic who evaluates the patient-doctor interaction and provides feedback on how to improve that interaction.

Many of us are worried about the prospect of medical AI. No doubt issues such as confidentiality, reliability, access ability and responsibility need to be addressed through debates within and beyond the medical profession. However, there are many amazing advantages – and many may be beyond our current imagination as this paper illustrates. To facilitate this debate and sharing of information, the *New England Journal of Medicine* has established a new (sponsored) interdisciplinary journal called *NEJM AI.* One of its first articles, by Hoifung Poon, Microsoft Research (2), describes the potential multimodal applications of medical AI.

References

1. Tu T, *et al*. Towards Conversational Diagnostic AI. *2401.05654 (arxiv.org)*
2. Poon H. Multimodal Generative AI for Precision Health. NEJM AI Sponsored. December 11, 2023. https://ai.nejm.org/doi/full/10.1056/AI-S2300233

## 15.12. Accurate proteome-wide missense variant effect prediction with AlphaMissense

Cheng J, Novati G, Pan J, Bycroft C, Zemgulyte A, Applebaum T, Pritzel A, Wong LH, Zielinski M, Sargeant T, Schneider RG, Senior AW, Jumper J, Hassabis D, Kohli P, Avsec Z.

*Science* **381**(6664): eadg7492 (2023).

PubMed: 37733863

In Brief: The authors describe AlphaMissense, a machine-learning tool that predicts the pathogenicity of 71 million human coding variants. 22.8 million variants (32%) are classified as likely pathogenic and 40.9 million (57%) as likely benign. They provide these databases freely as resources to the international research community.

These authors from Google DeepMind previously developed and released AlphaFold, a revolutionary machine-learning approach to predict 3-dimensional protein structures from 1-dimensional amino acid or gene sequence information – that was highlighted in an earlier Yearbook chapter (https://www.espeyearbook.org/ey/0019/ey0019.15-15). Here, they build on AlphaFold by incorporating evolutionary constraint information to predict the pathogenicity of all 216 million possible single amino acid changes in the 19 233 canonical human proteins. They evaluated the performance of their predictions against diverse clinical benchmarks, including against 18 924 annotated missense variants in ClinVar, and achieved an impressively high overall performance, with an receiver operator curve (auROC) of 0.940.

When we perform genetic sequencing in our patients, some will receive a confident clinical diagnosis due to having a known pathogenic mutation that is listed in an agreed international database such as ClinVar. However, and more typically, some will have 'variants of uncertain significance'. Indeed, the vast majority of missense variants detected on sequencing are of unknown clinical significance. In research studies, this presents an enormous burden for experimental functional assays to help inform which mutations are relevant. Even if such assays are performed, the cell-based functional readout may not correspond directly with the variant's clinical impact.

AlphaMissense will greatly accelerate this process. Although it can't (yet) be used directly in the context of clinical diagnosis, it will help in filtering which mutations to consider for functional validation and also for confirmatory analyses in large population-based biobanks.

## New Paradigms

## 15.13. Safety of low weight gain or weight loss in pregnancies with class 1, 2, and 3 obesity: a population-based cohort study

Johansson K, Bodnar LM, Stephansson O, Abrams B, Hutcheon JA.
*Lancet* **403**(10435): 1472–1481 (2024).
PubMed: 38555927

In Brief: The population-based Stockholm-Gotland Perinatal Cohort study analysed electronic medical records on 15 760 pregnancies with obesity, followed up for a median of 7.9 years. In pregnancies with class 1 or 2 obesity, low gestational weight gain (GWG, mean 0 kg at 40 weeks) did not increase risk of the adverse composite outcome (class 1: adjusted RR 0.97 [95% CI 0.89–1.06]; class 2: 0.96 [0.86–1.08]). In pregnancies with class 3 obesity, low GWG (mean 0 kg at 40 weeks) was associated with reduced risk of the adverse composite outcome (adjusted RR 0.81 [0.71–0.89]).

There are sparse data to inform the limits of healthy GWG. Possibly the only national guidelines (since used by several other countries) were published in 2009 by the US Institute of Medicine (IOM, currently the National Academy of Medicine). Those were based on risks for maternal postpartum weight retention, cesarean delivery, size at birth (SGA and LGA), preterm birth and childhood obesity. They stated that for women with pre-pregnancy obesity, GWG should be 11–20 pounds (5–9 kg), which was less than the previous IOM 1990 recommendation of 'at least 15 pounds' (~7 kg).

However, many women now start pregnancy with extremely high body mass index (BMI), and we now know this has a much larger impact than GWG on risks of gestational diabetes, pre-eclampsia and other adverse pregnancy outcomes. Importantly, it is currently unknown whether such women can safely avoid GWG or even reduce weight during pregnancy. The 2009 IOM committee acknowledged that most of their available data were on women with only class 1 obesity (BMI 30–34.9) and they had very few who showed GWG <5 kg.

This current large Swedish study fills this crucial evidence gap. Among women with pre-pregnancy class 1, class 2 (BMI 35–39.9), or class 3 (40+) obesity, 13.9%, 24.9%, and 33.2%, respectively, showed GWG below the 2009 IOM lower limit (5 kg). Furthermore, they extended the range of adverse outcomes beyond those considered by the 2009 IOM, to also include stillbirth, infant death, gestational diabetes, pre-eclampsia, and

new-onset longer-term maternal cardiometabolic disease after pregnancy, and weighted these to reflect their severity.

These findings will hopefully support new guidelines to lower or remove the lower limit of GWG for pregnant women with obesity and enable appropriate dietary and lifestyle advice.

## 15.14. Continuous glucose monitoring and intrapersonal variability in fasting glucose

Shilo S, Keshet A, Rossman H, Godneva A, Talmor-Barkan Y, Aviv Y, Segal E.
*Nat Med* **30**(5): 1424–1431 (2024).
PubMed: 38589602

In Brief: The authors investigated intraperson variability in fasting glucose (FG) levels using continuous glucose monitoring (CGM) devices in 8315 nondiabetic adults aged 40–70 years. Day-to-day intraperson standard deviation of FG was 7.52 mg/dl (0.42 mol/l). This high variability has a large estimated impact on the classification of glycemic status (normal FG, prediabetes or diabetes). Of 5328 adults classified as having normal FG based on their Day 1 measurement, 40% and 3% would be reclassified to prediabetes and diabetes, respectively, based on subsequent measurements.

It is likely that many of the diagnostic tests used in endocrinology and diabetes have poor reproducibility. This is likely due to physiological variation, as well as changes in preceding diets and behaviours. The use of repeated testing is limited by resources and participant burden. CGM provides access to several orders of magnitude more data than our traditional single clinic measurement for FG. FG was appropriately defined as measurements during the morning (between 0600 h and 0900 h) after a minimum 8 h fasting. Notably, while duration of fasting varied from 8 to 12 h, this was not correlated with FG values (Pearson, $r = 0.02$).

Of the 5328 adults classified as having normal FG by their Day 1 measurement, only 3030 (57%) also had all other FG measurements in the normal range. Individuals with higher FG variability (FG variance) reported diets with higher % carbohydrates, but had no differences in BMI, waist circumference, body fat or other cardiometabolic parameters.

The authors caution against basing diagnoses on only 1 or 2 FG values, and suggest use of CGM data to provide more reliable glycemic status assessment. As increasing diagnoses of diabetes and prediabetes are made based on HbA1c values, similar assessment of its reproducibility are needed.

## 15.15. Genetic drivers of heterogeneity in type 2 diabetes pathophysiology

Suzuki K, Hatzikotoulas K, Southam L, et al.
*Nature* **627**(8003): 347–357 (2024).
PubMed: 38374256

In Brief The authors report a genome-wide association study (GWAS) on Type 2 diabetes (T2D), including data from 2 535 601 individuals (39.7% non-European ancestry), including 428 452 with T2D. They identify 1,289 independent GWAS signals (at $P < 5 \times 10^{-8}$), of which 145 loci are novel. These genetic signals cluster into 8 groups, with differing cardiometabolic trait associations and differing cell-specific profiles of gene activation (open chromatin), including pancreatic islets, adipocytes, endothelial cells and enteroendocrine cells.

This paper reports the largest (GWAS) study to date of the common genetic determinants of T2D, generated through a new international collaboration with a nearly 3-fold larger sample size than previously. They combine the findings with single-cell data derived from disease-relevant tissues. Five genetic clusters were similar to previous reports: 1) beta-cell dysfunction positively or 2) negatively related with proinsulin, and also insulin resistance via 3) obesity, 4) lipodystrophy, or 5) liver and lipid metabolism. Three new clusters associated with cardiometabolic profiles of 1) metabolic syndrome, 2) body fat and 3) residual glycaemic effects.

GWAS for several traits have now reached the milestone of finding ~1000 independent common genetic signals. A major aspect of such studies is their power to separate different biological processes that contribute to the disease in question. This also informs us that different individuals may have developed the same disease but through differing predominant patho-aetiologies, e.g. beta cell dysfunction, general obesity, fat distribution, or other pathways to insulin resistance. It supports the prospect of precision medicine – that the optimal treatments and preventive strategies might be tailored to each individual.

The differing genetic clusters also showed differing strengths of association with other complex metabolic diseases. Gestational diabetes was more strongly related to beta cell dysfunction and lipodystrophy, whereas polycystic ovary syndrome was related more to obesity-related insulin resistance. Another recent genetics study portrayed youth-onset T2D also as a heterogeneous disease, but with a greater burden of rare mutations, and hence appears to be on a spectrum between monogenic diabetes and adult-onset T2D (1).

Reference
1. Kwak SH, et al. Genetic architecture and biology of youth-onset type 2 diabetes. *Nature Metabolism* volume 6, pages226–237 (2024)

## 15.16. Causality-enriched epigenetic age uncouples damage and adaptation

Ying K, Liu H, Tarkhov AE, Sadler MC, Lu AT, Moqri M, Horvath S, Kutalik Z, Shen X, Gladyshev VN.
*Nat Aging* **4**(2): 231–246 (2024).
PubMed: 38243142

In Brief: The authors harness large-scale genetic and DNA methylation datasets in an epigenome-wide Mendelian randomization approach, to identify CpG sites that appear to be causally related to aging-related traits. These sites are collated to produce 2 epigenetic clocks: DamAge has adverse impacts on age-related outcomes, including mortality. AdaptAge appears to confer beneficial adaptations.

Steve Horvath and others originally described various epigenetic clocks based on robust age-related changes in DNA methylation that correspond closely to chronological age in a highly reproducible pattern. For example, such tools can be used in forensics to estimate the age of a person by their crime-scene DNA sample with an error margin as low as 2–5 years, or to identify individuals who exhibit an accelerated ageing trajectory. The ambition has now moved beyond prediction to causation – to identify epigenetic changes that have a causal impact on ageing. With that aim, Horvath and others established Altos Labs, whose funders include Jeff Bezos the founder of Amazon, with the goal of developing therapies to reverse aging and extend human lifespan.

In contrast to previous phenotypic observation-based approaches, here they used Mendelian randomization (MR), a genetic causal inference analysis approach that reduces the possibilities of confounding and reverse causality. Hence MR produces more robust evidence than that from typical phenotypic observations. DamAge showed remarkably strong inverse correlation with time of cell reprogramming ($R = -0.93$, $P = 4 \times 10 - 12$) when mature fibroblasts were artificially converted to induced pluripotent stem cells (iPSC), indicating that these methylation changes have closely synergy with cell 'stemness' factors. Advanced DamAge was also seen in the rare ageing disorders Hutchinson–Gilford progeria and Werner syndrome, and in children conceived by IVF with fresh or cryopreserved embryos, but was surprisingly reduced in children born with low birth weight (SGA).

In currently available human genetic and DNA methylation datasets, only ~10% of DNA methylation sites (CpG sites) measured by arrays have a robust genetic signal (meSNP) that can be used in MR studies to model the causal impacts of changes in methylation. As such source datasets grow, this type of approach will increase substantially in power and coverage.

# Author Index