Yearbook of Paediatric Endocrinology 2022

Editors
Ken Ong
Christa Flück

published by
bioscientifica

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Paediatric Endocrinology 2022

Endorsed by the European Society for Paediatric Endocrinology

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

Typeset by OKS Prepress

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Preface

This year is my first year in the team of the ESPE Yearbook writers in the role as co-editor (CF). As honored as I was when offered this post last year, as afraid I was in the past weeks when facing the task. Although I have been co-author of the Adrenal Chapter and then the lead of the Chapter on Differences of Sexual Development and Gender Dysphoria for the last 5 years, overlooking the whole Yearbook project seemed a challenging task. But having Ken Ong on my side, I realized that he is actually in full control, very experienced and extremely efficient. He made my start into the new role as a co-editor for the ESPE Yearbook easy and enjoyable, while taking extra efforts with a newcomer on his side. Therefore, I would like to thank Ken and give him the extra credit he deserves by writing these words.

We are indebted to Ze'ev Hochberg (Haifa) who originated and coedited the Yearbook since its first edition in 2004 until last year, and we wish him a long and happy retirement. We also thank ESPE, Bioscientifica and all of the Associate Editors and their teams for their crucial roles in supporting the Yearbook. In our ongoing quest to improve the content and format of the Yearbook, we welcome your comments and suggestions.

Christa E. Flück and Ken K. Ong

1. Pituitary and Neuroendocrinology

Yafei Wang[1], Anna-Pauliina Iivonen[1], Johanna Hietamäki[2], Juho Kärkinen[2], Matti Hero[2], Päivi J. Miettinen[2], Taneli Raivio[1,2]

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Basic Science and Stem Cells

1.1. Deciphering the spatial-temporal transcriptional landscape of human hypothalamus development

Zhou X, Lu Y, Zhao F, Dong J, Ma W, Zhong S, Wang M, Wang B, Zhao Y, Shi Y, Ma Q, Lu T, Zhang J, Wang X, Wu Q
Cell Stem Cell. 2022 Feb 3;29(2):328-343.e5.
doi: 10.1016/j.stem.2021.11.009. PMID: 34879244.

Brief Summary: By applying single-cell RNA sequencing (scRNA-seq) to 112,376 cells of human hypothalamus ranging from 7–20 gestational weeks (GW7–20), the authors produced a spatiotemporal transcriptome atlas of human hypothalamus development and revealed critical regulatory genes controlling the cell-fate decisions of neuroepithelial cells and neural progenitors.

The hypothalamic regulation of endocrine, autonomic, and behavioural functions depend on various cell types and nuclei. However, developmental characterization of the human hypothalamus has been less investigated.

This work generated scRNA-seq dataset based on human hypothalamus cells ranging from 7-20 gestational weeks (GW7-20), which illustrated spatiotemporal transcriptional characteristics of cell types and their differentiation lineage in developing human hypothalamus. These results showed that early neuroepithelium and progenitors at different hypothalamus locations with distinctive potential cell fates show molecular heterogeneity and that cell fate might be determined as early as at GW7. The authors identified the regulator genes controlling the cell-fate (gliogenic or neurogenic) determination of neural progenitors, figured out the temporal windows and spatial ordering for the formation of different nuclei in the human hypothalamus, and revealed molecular diversification of arcuate nucleus neuron subpopulations. In addition, they investigated the divergence or consistency of cell-type relationships in human and mice, and found that the human hypothalamic gliogenesis occurs at an earlier stage of gestation and displays distinctive transcription profiles compared with those in mice. Notably, early oligodendrocyte cells in humans exhibit different gene patterns and interact with neuronal cells to regulate neuronal maturation by Wnt, Hippo, and integrin signals. This scRNA-seq dataset from developing human hypothalamus provides a deep understanding of the mechanisms of cellular network organization, circuit formation, and hypothalamic dysfunction.

1.2. Cis-regulatory architecture of human ESC-derived hypothalamic neuron differentiation aids in variant-to-gene mapping of relevant complex traits

Pahl MC, Doege CA, Hodge KM, Littleton SH, Leonard ME, Lu S, Rausch R, Pippin JA, De Rosa MC, Basak A, Bradfield JP, Hammond RK, Boehm K, Berkowitz RI, Lasconi C, Su C, Chesi A, Johnson ME, Wells AD, Voight BF, Leibel RL, Cousminer DL, Grant SFA
Nat Commun. 2021 Nov 19;12(1):6749.
doi: 10.1038/s41467-021-27001-4. PMID: 34799566.

Brief Summary: The authors used RNA-seq, ATAC-seq, and promoter-focused Capture C to characterize the genetic architecture during hypothalamic differentiation. This information provides insights into mechanisms by which noncoding GWAS loci are associated with hypothalamic-regulated traits potentially mediating their effects.

The hypothalamus controls numerous complex traits, such as timing of sexual maturation and body weight. Understanding of the traits and related diseases could be increased by studying the genetic regulation of the developing and mature human hypothalamus. However, availability of human hypothalamic tissue is limited. Moreover, in genome-wide association studies (GWAS), most signals associated with complex traits lie in the non-coding regions of the genome, indicating that they can be in *cis* -acting regulatory elements of gene (for instance, enhancers or silencers). Since *cis* -acting regulatory elements can act locally or over large distances, the closest gene to a GWAS signal may not be the main effector gene.

This study generates a high-resolution chromatin architecture atlas of an established embryonic stem cell -derived hypothalamic-like neuron model across three stages of *in vitro* differentiation. The authors profile open chromatin and identify physical contacts between gene promoters and putative *cis* -regulatory elements in order to characterize the global regulatory landscape changes during hypothalamic differentiation. The data were integrated with GWAS signals for complex traits, including body mass index, adult height, sleep, age at menarche, and psychiatric disorders. This approach pinpointed both known and novel effector genes along with their corresponding putative regulatory elements for the traits. A further biological function analysis revealed the association of the potential effector genes with central pathways for hypothalamic development.

Although a cell model is not fully comparable to *in vivo* conditions, this paper provides valuable data for prioritizing the candidate genes that drive the hypothalamic molecular mechanisms influencing the pathogenesis of complex traits.

1.3. Generation of hypothalamic arcuate organoids from human induced pluripotent stem cells

Huang WK, Wong SZH, Pather SR, Nguyen PTT, Zhang F, Zhang DY, Zhang Z, Lu L, Fang W, Chen L, Fernandes A, Su Y, Song H, Ming GL

Cell Stem Cell. 2021 Sep 2;28(9):1657-1670.e10.

doi: 10.1016/j.stem.2021.04.006. PMID: 33961804.

Brief Summary: The authors established a novel protocol to generate hypothalamic arcuate organoids from human induced pluripotent stem cells, which could be utilized to investigate the arcuate nuclei development and the underlying mechanism of arcuate nucleus-related diseases.

Human three-dimensional (3D) brain organoids represent remarkable platforms for recapitulating features of human brain development and diseases. These 3D organoid models involve cell-cell and cell-matrix interactions and create better biomimetic tissue models than two-dimensional models. However, existing organoid models do not resolve fine brain subregions, such as different nuclei in the hypothalamus.

This study reported a protocol of generating arcuate organoids (ARCOs) from human induced pluripotent stem cells (iPSCs) to model the development of the human hypothalamic arcuate nucleus. These ARCOs exhibit similar cell type diversity and molecular signatures compared with the human arcuate nucleus (ARC) on single cell RNA sequencing analysis. The authors used this model to explore the development and function of ARCOs derived from Prader-Willi syndrome patient iPSCs (derived from fibroblasts). They found that patient iPSC-derived ARCOs exhibit aberrant differentiation and decreased leptin responses compared with control ARCOs. Furthermore, transcriptome analysis indicates that the patient-derived ARCOs show increased immune responses.

Thus, ARCOs derived from human iPSCs can be used to model early hypothalamic ARC developmental processes and investigate the mechanism of related brain diseases.

1.4. Pituitary stem cells produce paracrine WNT signals to control the expansion of their descendant progenitor cells

John P Russell, Xinhong Lim, Alice Santambrogio, Val Yianni, Yasmine Kemkem, Bruce Wang, Matthew Fish, Scott Haston, Anaëlle Grabek, Shirleen Hallang, Emily J Lodge, Amanda L Patist, Andreas Schedl, Patrice Mollard, Roel Nusse, Cynthia L Andoniadou

Elife. 2021 Jan. 10:e59142.
doi: https://doi.org/10.7554/eLife.59142.

Brief Summary: The authors studied genetic mice models to show that pituitary stem cells can secrete WNT ligands to their committed progeny and promote their expansion.

The anterior pituitary contains a population of *Sox2* expressing stem cells (Sox2+ PSCs), which self-renew and give rise to lineage-committed progenitors and functional endocrine cells. The WNT pathway is upregulated during growth and regeneration, which raises the question about the relationship between Sox2+ PSCs and Wnt signaling.

Using the WNT signaling activity reporter mice line, the authors noticed that all the different cell types of the anterior pituitary are WNT-responsive. Through lineage tracing, they found that activation of the WNT pathway is necessary for the expansion of the pituitary populations. By checking the gene expression profiles of Sox2+ and Sox2- cells, they found that Sox2+ PSCs express WNT ligands as well as essential components regulating activation of the WNT pathway. Furthermore, they used another genetic mouse model to block WNT secretion specifically in Sox2+ PSCs, and found that it reduced the proliferation of pituitary cells.

This study discovered a novel function of Sox2+ PSCs to drive the expansion of committed progenitors through secreting WNT ligands and is of relevance to understanding development, disease, regeneration, aging, and cancer of the pituitary gland.

Genetics

1.5. Mutations in HID1 Cause Syndromic Infantile Encephalopathy and Hypopituitarism

Schänzer A, Achleitner MT, Trümbach D, Hubert L, Munnich A, Ahlemeyer B, AlAbdulrahim MM, Greif PA, Vosberg S, Hummer B, Feichtinger RG, Mayr JA, Wortmann SB, Aichner H, Rudnik-Schöneborn S, Ruiz A, Gabau E, Sánchez JP, Ellard S, Homfray T, Stals KL, Wurst W, Neubauer BA, Acker T, Bohlander SK, Asensio C, Besmond C, Alkuraya FS, AlSayed MD, Hahn A, Weber A

Ann Neurol. 2021 Jul;90(1):143-158.
doi: 10.1002/ana.26127. PMID: 33999436.

Brief Summary: This study identifies biallelic *HID1* variants in 7 patients with hypopituitarism and infantile encephalopathy. It provides genetic and functional evidence for a novel gene-disease connection and expands the list of central nervous system diseases caused by impairment of the trans-Golgi network.

The authors describe 7 infants, 3 of whom had sadly died, with developmental delay, seizures, thin corpus callosum, brain atrophy, and hypopituitarism. The authors used whole exome sequencing to reveal the genetic cause and discovered that every patient harbored segregating, biallelic *HID1* variants. The variants were either missense and likely pathogenic (according to the American College of Medical Genetics (ACMG) criteria), frameshifts leading to a premature STOP codon, or located at a splice site. One of the variants was shown to reduce the extracellular acidification rate upon potassium chloride stimulation, which indicated impaired function of the trans-Golgi network.

The trans-Golgi network and *HID1* (HID1 domain containing) are closely connected, for HID1 participates in the formation of neuropeptide- and peptide hormone-loaded vesicles via acidification of the network, a vital step for the secretion of the loads (1-3). They demonstrated the highest expression of *HID1* in the brain and secretory tissues, especially in the pituitary in humans, and an increase in the brain expression with gestational age. They thus speculated that *HID1* could play a role in brain development and the function of the neuronal and secretory cells. In fact, *HID1* has been previously proposed to be a candidate gene for syndromic hypopituitarism (4), but no functional evidence on its connection to this phenotype had been provided.

In summary, these findings suggest that *HID1* variants underlie a severe form of syndromic hypopituitarism, and that defects in this gene should be considered in the molecular genetic diagnosis of cases with early infantile encephalopathy and multiple pituitary hormone deficiencies.

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1.6. High-throughput splicing assays identify missense and silent splice-disruptive POU1F1 variants underlying pituitary hormone deficiency

Gergics P, Smith C, Bando H, Jorge AAL, Rockstroh-Lippold D, Vishnopolska SA, Castinetti F, Maksutova M, Carvalho LRS, Hoppmann J, Martínez Mayer J, Albarel F, Braslavsky D, Keselman A, Bergadá I, Martí MA, Saveanu A, Barlier A, Abou Jamra R, Guo MH, Dauber A, Nakaguma M, Mendonca BB, Jayakody SN, Ozel AB, Fang Q, Ma Q, Li JZ, Brue T, Pérez Millán MI, Arnhold IJP, Pfaeffle R, Kitzman JO, Camper SA

Am J Hum Genet. 2021 Aug 5;108(8):1526-1539.
doi: 10.1016/j.ajhg.2021.06.013. PMID: 34270938.

Brief Summary: This study reports splice-disruptive variants in *POU1F1* in 4 families with hypopituitarism and uses a high-throughput splicing reporter assay to create a comprehensive catalogue of such variants in or near exon 2 of the gene. The catalogue paves the way for identifying synonymous but splice-disruptive *POU1F1* variants in additional patients with hypopituitarism, which underscores the importance of evaluating defective splicing as a disease mechanism.

Defects in *POU1F1* are well-known causes of hypopituitarism (1). The gene undergoes alternative splicing, to produce the main alpha and the minor beta isoform that act as a transcriptional activator and repressor, respectively. The so far reported dominant-negative *POU1F1* variants are located in domains shared by both isoforms and have been functionally tested only for the alpha isoform.

Here, the authors report 4 families with hypopituitarism and heterozygous missense variants in exon 2 of the *POU1F1* beta isoform. Functional assays showed that the variants shifted splicing to the production of the beta isoform, which causes dominant-negative loss-of function by suppressing the alpha isoform. A high-throughput splicing reporter assay was used to measure the impact of 1070 single-nucleotide variants in *POU1F1* exon 2 and flanking intronic regions on splicing. This approach revealed 96 splice-disrupting variants of which 14 were synonymous. Two of the synonymous variants were present in 2 additional, unrelated patients with hypopituitarism and both increased the beta isoform usage.

This study reminds us about the importance of evaluating the effects of variants, including the coding ones, on splicing. Moreover, it introduces a splicing effect catalogue of *POU1F1* variants and highlights the increase of the beta isoform usage as a consequence of splice-disruptive variants in this gene.

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1.7. Pathogenic variants in *RNPC3* are associated with hypopituitarism and primary ovarian insufficiency

Akin L, Rizzoti K, Gregory LC, Corredor B, Le Quesne Stabej P, Williams H, Buonocore F, Mouilleron S, Capra V, McGlacken-Byrne SM, Martos-Moreno GÁ, Azmanov DN, Kendirci M, Kurtoglu S, Suntharalingham JP, Galichet C, Gustincich S, Tasic V,

Achermann JC, Accogli A, Filipovska A, Tuilpakov A, Maghnie M, Gucev Z, Gonen ZB, Pérez-Jurado LA, Robinson I, Lovell-Badge R, Argente J, Dattani MT
Genet Med. 2022 Feb;24(2):384-397.
doi: 10.1016/j.gim.2021.09.019. PMID: 34906446.

Brief Summary: This study extends the phenotypes related to pathogenic biallelic *RNPC3* variants to cover primary ovarian insufficiency (POI) in combination with the previously associated growth hormone deficiency (GHD).

The authors report 15 patients from 9 pedigrees with severe GHD, hypoprolactinemia, variable thyrotropin (TSH) deficiency, and also POI in 8/9 affected females. POI manifested as stalled puberty, whereas affected males had normal pubertal development and gonadal function. Severe biochemical GHD was present in all patients, but 2 male patients exhibited normal growth while off exogenous growth hormone supplementation. Anterior pituitary hypoplasia was noted in 13 patients, and 4 patients from different pedigrees were diagnosed with motor neuropathy.

Next generation sequencing, followed by Sanger sequencing, revealed biallelic pathogenic-assessed variants in *RNPC3* in all patients, including a novel homozygous missense variant c.1449A>T (p.Leu483Phe) in 8 patients from 5 consanguineous Turkish pedigrees. *RNPC3* encodes a protein component of minor spliceosome and represents a novel genetic mechanism causative for POI.

Using in situ hybridization, the authors showed that *RNPC3/Rnpc3* is expressed in multiple central nervous system areas, including the hypothalamus and Rathke's pouch in both human and mouse. *Rnpc3* was also expressed in the ovaries of adult mice. The data were partially supported by the phenotype of CRISPR-Cas9 generated *Rnpc3* $^{\text{p.Leu483Phe/p.Leu483Phe}}$ mouse model: the female, but not male mice had GHD. There was no sign of POI in young mice.

To summarize, this study describes patients with biallelic pathogenic *RNPC3* variants, manifesting with variable pituitary hormone deficiency, and POI. Indeed, the disruption of both central and peripheral endocrine functions is quite unusual in monogenic endocrine disorders. Future functional studies are needed to elucidate the role of *RNPC3* variants for the motor neuropathy phenotype.

1.8. Intronic variant in *POU1F1* associated with canine pituitary dwarfism

Kyöstilä K, Niskanen JE, Arumilli M, Donner J, Hytönen MK, Lohi H
Hum Genet. 2021 Nov;140(11):1553-1562.
doi: 10.1007/s00439-021-02259-2.

Brief Summary: This paper describes 5 related Karelian Bear Dogs (KBDs) with a canine hypopituitarism phenotype. Genome-wide association analysis (GWAS) and next-generation sequencing revealed a homozygous candidate gene defect in *POU1F1*. The study thus presents a novel animal model for human hypopituitarism.

In addition to humans, hypopituitarism can spontaneously occur in animals, including mice and dogs. In dogs, hypopituitarism has been reported in German Shepherds and KBDs. The affected dogs of both breeds manifest with small size and puppy coats with hair loss (1, 2). The German Shepherds have deficiencies of growth hormone, thyroid-stimulating hormone, gonadotropins, and prolactin due to a recessive splicing variant in *LHX3* (1, 3), whereas the KBDs have low insulin-like growth factor 1 levels and a suggested autosomal recessive disease (2).

Here, the authors describe 5 related KBDs, who were suspected of hypopituitarism based on their size and coat. In addition, 1 of these dogs had a low level of insulin-like growth factor and 1 had hypothyroidism. GWAS using SNP array data, performed in the 5 affected cases and 139 KBD controls, mapped the disease locus to chromosome 31, where the affected dogs shared allelic homozygosity. Whole genome sequencing on 3 affected dogs filtered the best candidate gene to a homozygous intronic variant in the gene, *POU1F1*. The variant was predicted to affect gene splicing and it was heterozygous or absent in 1035 control genomes or exomes of various breeds.

Genotyping the variant in a cohort of 642 KBDs, including the affected cases and their available close relatives, revealed 62 heterozygotes and 3 additional homozygous individuals, 1 of which was still alive and manifested a hypopituitarism phenotype. Furthermore, 7 Lapponian Herders harboring the *POU1F1* variant were identified among 349 dogs of related breeds and 1 heterozygote KBD among 7925 dogs from 206 different breeds, indicating high KBD breed-specificity of the *POU1F1* variant.

To conclude, this paper suggests that a rare homozygous defect in *POU1F1* can underlie hypopituitarism not only in humans (4) but also in dogs. However, future efforts are needed to comprehensively evaluate the canine hormone deficiencies and show the functional effect of the variant on splicing.

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1.9. Uncertain, Not Unimportant: Callosal Dysgenesis and Variants of Uncertain Significance in *ROBO1*

Woodring TS, Mirza MH, Benavides V, Ellsworth KA, Wright MS, Javed MJ, Ramiro S.

Pediatrics. 2021 Jul;148(1):e2020019000.

doi: 10.1542/peds.2020-019000.

Brief Summary: This case report of an infant with dysgenesis of the corpus callosum, whose whole genome sequencing (WGS) revealed variants of unknown significance (VUS) in *ROBO1,* demonstrates the potential of VUS in guiding clinical decisions.

Next-generation sequencing (NGS) is increasingly applied in investigations of suspected genetic diseases (1). Only a subset of the interesting variants identified by NGS are further evaluated and classified as pathogenic, likely pathogenic, likely benign, or benign according to the American College of Medical Genetics (ACMG) classification (2, 3). In fact, in as many as 70% of cases, NGS cannot identify a pathogenic variant for diagnosis (4). However, when genetic disease is highly suspected, careful evaluation of the available evidence might show that VUS are relevant for the case in question.

Here, the authors present a male infant who presented with small size for gestational age, dysmorphic face, hypospadias, and retractile testes. Head ultrasound revealed hydrocephalus, absent septum pellucidum, dysgenesis of the corpus callosum, and a finding suggestive of Probst bundles, aberrant bundles of axons running in an anterior-posterior direction between the cerebral hemispheres. These notions raised the suspicion of a genetic axon guidance disorder. He underwent rapid WGS, which revealed 2 rare VUS at conserved sites in the axon guidance receptor gene, *ROBO1*.

Due to the previous reported associations of *ROBO1* variants with dysgenesis of midline structures, renal abnormalities, and hypopituitarism, the patient was further examined. The subsequent workup confirmed the callosal defect and uncovered kidney anomalies, as well as pituitary dysfunction. Finally, to support other investigators in critical evaluation of VUS, the paper presents a set of online resources for this purpose.

To conclude, this case report exemplifies how individualized interpretation of VUS as pathogenic may lead to early detection of accompanying anomalies and guide treatment. The paper also shows that the clinical judgment of variant pathogenicity can differ from and replace the ACMG classification. Importantly, this report sets a reminder of updating the variant interpretation as evidence accumulates.

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Clinical Papers

1.10. Genetics, clinical features and outcomes of non-syndromic pituitary gigantism: experience of a single center from Sao Paulo, Brazil

Trarbach EB, Trivellin G, Grande IPP, Duarte FHG, Jorge AAL, do Nascimento FBP, Garmes HM, Nery M, Mendonca BB, Stratakis CA, Bronstein MD, Jallad RS

Pituitary. 2021;24(2):252-261.
doi: 10.1007/s11102-020-01105-4. PMID: 33156432.

Brief Summary: Pituitary gigantism is a rare disease, which can be syndromic, as in McCune-Albright syndrome, Carney complex, *MEN1* and *MEN4*, and the newly described "three P association" (paraganglioma, pheochromocytoma and pituitary adenoma) (1), or non-syndromic caused by mutations in aryl hydrocarbon receptor-interacting protein (*AIP*) or microduplication of Xq26.3 in X-linked acrogigantism i.e. X-LAG (2). This study reports a cohort of non-syndromic pituitary gigantism.

The authors describe a single center experience of non-syndromic patients. Their cohort comprised 18 patients from Sao Paolo, 4 of whom had pathogenic variants in *AIP* gene and 2 had X-LAG. The median age at pituitary gigantism diagnosis was 17 [range 15-20] years but the first signs and symptoms were noted 3.5 years earlier (median 14 [range 9-16] years). The X-LAG patients were diagnosed earlier than those with no identified mutations. However, as no growth charts were available, the authors could not verify the previously reported very early onset height acceleration. All patients had elevated levels of GH and IGF1. Pituitary tumors were macroadenomas and positive for GH immunoreactivity.

On top of the clinical description of pituitary gigantism, the paper emphasizes the importance of regular growth measurements and use of growth charts as simple and cheap tools in the detection of this rare disease.

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1.11. Duplications disrupt chromatin architecture and rewire *GPR101*-enhancer communication in X-linked acrogigantism

Franke M, Daly AF, Palmeira L, Tirosh A, Stigliano A, Trifan E, Faucz FR, Abboud D, Petrossians P, Tena JJ, Vitali E, Lania AG, Gómez-Skarmeta JL, Beckers A, Stratakis CA, Trivellin G

Am J Hum Genet. 2022;109(4):553-570. PMID: 35202564.
doi: 10.1016/j.ajhg.2022.02.002.

Brief Summary: The authors present elegant data showing that X-LAG is a TADopathy of the endocrine system and that the rewiring of *GPR101* -enhancer interactions most likely causes the upregulation of *GPR101* expression in X-LAG-related pituitary tumors.

The X-LAG microduplication Xq26.3 contains the genes *VGLL1*, *CD40LG*, *ARHGEF6*, *RBMX*, and *GPR101*. *GPR101* encodes a class A rhodopsin-like orphan G-protein coupled receptor. Pituitary tumors overexpress *GPR101*, but the exact mechanism leading to this has been unclear. Here, the authors suggest this is due to rewiring of regulatory elements and formation of a new topologically associated domain (TAD). TADs are most likely a new concept to us pediatric endocrinologists. Generally, temporospatial expression of genes is mediated by cis-regulatory elements (CREs), *a.k.a.* promoters and enhancers, and by transcription factors. To simplify, the genome is divided into discrete regulatory units, named TADs, which take part in regulating the specificity of enhancer-promoter interactions. TADs are separated from each other by TAD borders, which are enriched by DNA-binding factor CTCF. It has been shown previously that the disruption of TADs through duplications, deletions, or insertions can rewire long-range regulatory architecture and result in pathogenic phenotypes. Examples of such diseases are Quebec platelet disorder and limb malformations. (1, 2)

The authors used Hi-C and 4C-seq methods in 6 patients with X-LAG and showed that *GPR101* lies within a TAD, separate from centromeric genes within the X-LAG locus. The microduplications in the Xq26.3 chromosomal region led to disruption of the TAD structure, demonstrated by ectopic interactions that crossed the TAD border. The rearrangement, caused by the duplications, forms a neo-TAD that exposes the *GPR101* promoter to interact with nearby cis-regulatory elements (CREs), *i.e.,* enhancer adoption within the new regulatory unit. Subsequently, the authors demonstrated *in vitro* that one of the identified pituitary CREs within the neo-TAD significantly enhanced reporter gene expression.

In summary, rewiring *GPR101* -enhancer interaction within the new regulatory unit is likely to cause the high levels of aberrant expression of *GPR101* in pituitary tumors seen in X-LAG. Therefore, X-LAG is actually a TADopathy.

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1.12. Dysgenesis and Dysfunction of the Pancreas and Pituitary Due to *FOXA2* Gene Defects

Kaygusuz SB, Arslan Ates E, Vignola ML, Volkan B, Geckinli BB, Turan S, Bereket A, Gaston-Massuet C, Guran T

J Clin Endocrinol Metab. 2021, 106(10):e4142-e4154.
doi: 10.1210/clinem/dgab352. PMID: 33999151.

Brief Summary: The authors show that patients with hypopituitarism and *FOXA2* gene defects also need screening for dysfunction of the pancreas.

The Forkhead box A2 transcription factor (*FOXA2*) is important for normal development of the central nervous system, including the pituitary gland, and also for endoderm-derived organs in concert with *e.g.* Sonic Hedgehog (*SHH*). Previously, Giri *et al.* (1) reported a patient with a heterozygous *FOXA2* mutation who had a complex congenital syndrome with hypopituitarism, hyperinsulinism, and endoderm-derived organ abnormalities. The mechanism of hyperinsulinemia remained unclear, although *FOXA2* mutations may downregulate *HADH* and thus affect beta oxidation, downregulate *GLUT2* and also KATP subunit expression.

The current study broadens the clinical *FOXA2* -related pancreatic phenotype. Although it describes only 2 patients with hypopituitarism, their pancreatic hypoplasia or sulphonyl-urea responsive diabetes are new clinical findings. The first patient had a novel heterozygous nonsense *FOXA2* variant, which encoded a truncated protein lacking part of the DNA-binding domain, and *in vitro* showed impaired transcriptional activation of *GLUT2* -luciferase. Besides neonatal onset hypopituitarism, he had neonatal hyperinsulinism and later developed impaired glucose tolerance. Pancreatic hypoplasia was apparent on CT scan and exocrine function was mildly reduced. The second patient had a novel *de novo* 8.53 Mb deletion encompassing *FOXA2*. He presented with progressive hypopituitarism at age 20 months and at age 16 years he developed diabetes, which was responsive to sulphonylurea treatment.

Another recent study (2) showed that inactivation of *FOXA2* in iPSCs downregulates key pancreatic development-specific transcription factors and explains the here detected pancreatic hypoplasia and dysfunction. Nevertheless, the mechanisms for neonatal hyperinsulinism and the progression to insulin-deficient diabetes remain open.

In conclusion, the authors propose that screening of *FOXA2* should be considered as the first step in managing patients with hypopituitarism and multiple endodermal-derived organ anomalies, and regular follow-up is mandatory.

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1.13. Management of children and young people with idiopathic pituitary stalk thickening, central diabetes insipidus, or both: a national clinical practice consensus guideline.

Cerbone M, Visser J, Bulwer C, Ederies A, Vallabhaneni K, Ball S, Kamaly-Asl I, Grossman A, Gleeson H, Korbonits M, Nanduri V, Tziaferi V, Jacques T, Spoudeas HA

Lancet Child Adolesc Health. 2021 Sep;5(9):662-676.
doi: 10.1016/S2352-4642(21)00088-2. Epub 2021 Jun 30; PMID: 34214482.

Brief Summary: This comprehensive guideline defines the role of cerebrospinal fluid tumor markers, whole-body imaging, and the indications, timing and risks of stalk biopsy, as well as criteria for discharge in children and young people with idiopathic pituitary stalk thickening, central diabetes insipidus, or both.

Unexplained pituitary stalk thickening and/or central diabetes insipidus (DI) in children and adolescents may be due to occult neoplasia, congenital defects, or may remain idiopathic in a third of the patients even after careful work-up. Differential diagnostics is often problematic.

This paper, by an expert UK national guideline development group, describes a systematically developed management flowchart and clinical practice guideline to inform specialist care and improve outcomes in children and young people (aged <19 years) with idiopathic pituitary stalk thickening, central diabetes insipidus, or both.

The authors recommend dynamic pituitary function testing, specialist pituitary imaging, measurement of serum β-human chorionic gonadotropin and alpha-fetoprotein concentrations, chest x-ray, abdominal ultrasonography, optometry, and skeletal survey for all such patients. Stalk thickening of 4 mm or more at the optic chiasm, 3 mm or more at pituitary insertion, or both, is potentially pathological, particularly if an endocrinopathy or visual impairment coexists. The authors also make recommendations regarding surveillance, the use of cerebrospinal fluid tumor markers, whole-body imaging, indications, timing and risks of stalk biopsy, and give criteria for discharge. Finally, they also encourage to set up registry studies to validate its recommendations.

This guideline serves as a valuable reference for pediatric endocrinologists and oncology colleagues who care for children with pituitary stalk thickening and/or DI.

1.14. The necessity of magnetic resonance imaging in the evaluation of pediatric growth hormone deficiency: Lessons from a large academic center

Mamilly L, Pyle-Eilola AL, Chaudhari M, Henry RK

Growth Horm IGF Res. 2021;60-61:101427.
doi: 10.1016/j.ghir.2021.101427. PMID: 34592640.

Brief Summary: This retrospective chart review describes abnormalities on Magnetic resonance imaging (MRI), according to severity of growth hormone deficiency (GHD) in children.

MRI of the pituitary gland is recommended following the diagnosis of GHD. In prior studies, provocative GH test results and MRI findings may not correlate well (1). However, it remains less clear whether the likelihood of discovering a serious etiology of GHD, such as a tumor, varies by peak GH level.

These authors investigated this question in a retrospective series of 399 children with GHD from their institute. In children with isolated GHD (IGHD) and multiple pituitary hormone deficiency (MPHD), pituitary abnormalities were more likely to be found in children with peak GH levels at the lowest tier of provocative test results (group A with peak GH $<$ 5.0 ng/mL) compared to higher levels of failed GH test results (group B with peak GH 5.0–7.4 ng/mL and group C with peak GH 7.5–9.9 ng/mL). In group A, 36.9% had an abnormal pituitary vs. 16.7% and 17.0% in groups B and C, respectively. In children with IGHD, abnormal MRI findings were most frequently found in those from group A (28.4%) in comparison to groups B and C (15.3% and 16.9%, respectively).

While these results showed that abnormal MRI findings may be present across all categories of failed GH provocative test results, it is important to note that findings in groups B and C were less frequently "actionable" and all patients with a tumor showed severe GHD (peak GH $<$ 5.0 ng/mL). The authors concluded that further research is needed to inform future guidelines on the necessity of imaging for cases with a GH peak of 5 – 9.9 μg/mL.

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2. Antenatal and Neonatal Endocrinology

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Neonatal Hypoglycaemia

2.1. Dextrose gel for neonates at risk with asymptomatic hypoglycemia: a randomized clinical trial

Gupta K, Amboiram P, Balakrishnan U, C A, Abiramalatha T, Devi U
Pediatrics. 2022 1;149(6):e2021050733.
doi: 10.1542/peds.2021-050733. PMID: 35582897.

Brief Summary: This randomized clinical trial in 'at-risk' neonates with asymptomatic hypoglycemia tested whether oral dextrose gel prevented the need for intravenous fluids. Oral dextrose gel reduced the need for intravenous fluids as well as admission to the neonatal intensive care unit (NICU).

Hypoglycemia is common in at risk neonates in the first a few days of life. Many of these neonates may require admission to the NICU for intravenous fluids to treat the hypoglycemia. Using oral 40% dextrose gel for treating hypoglycemia may be one way to reduce the need for admission to NICU and intravenous fluids and prevent separation of infant from the mother (1). Its use in neonates has not been associated with any adverse effect until 2 years of age.

In this randomized clinical trial in neonates in high risk groups for hypoglycaemia who presented with asymptomatic hypoglycemia, oral dextrose gel promoted exclusive breastfeeding, and reduced mother-infant dyad separation in the first 48 hours of life. It was effective individually across 3 categories of risk groups, namely small for gestational age infants and intrauterine growth retarded infants, insulin dependent diabetic infants and large for gestational age infants, and late preterm infants.

This trial showed that oral 40% dextrose gel followed by breast feeding (compared to breast feeding alone) was a cost-effective alternative to intravenous fluids for the management of asymptomatic hypoglycemia in at-risk neonates. Oral dextrose gel was not associated with any adverse effects and was readily accepted by the mothers involved in the study. The use of continuous glucose monitoring in this study would have potentially provided more accurate and real time glucose measurements.

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2.2. Oral dextrose gel for the treatment of hypoglycaemia in newborn infants

Edwards T, Liu G, Battin M, Harris DL, Hegarty JE, Weston PJ, Harding JE
Cochrane Database Syst Rev. 2022 Mar 18;3(3):CD011027.
doi: 10.1002/14651858.CD011027.pub3. PMID: 35302645.

Brief Summary: This systematic review assessed the evidence for oral dextrose gel to treat hypoglycemia in newborns. Oral dextrose gel compared with placebo gel *probably* improved the correction of hypoglycaemic events and may result in a slight reduction in the risk of major neurological disability at 4.5 years corrected age, but the evidence is still uncertain.

Neonatal hypoglycaemia is a common condition affecting about 5% to 15% of infants in the immediate postnatal period, especially in the at-risk group pf infants such as the IUGR, preterm and the infants of diabetic mothers. It can be associated with brain injury, developmental problems and poor later school performance. Once diagnosed the management involves increased feeding, supplemental infant formula or intravenous dextrose. Supplemental infant formula may disrupt the establishment of breastfeeding. Intravenous dextrose is expensive, usually requires separation of mother and infant and is not always available in resource-poor settings, or settings providing lower levels of perinatal care.

Oral dextrose gel is now widely used, and is increasingly recommended as a first-line treatment for asymptomatic neonatal hypoglycaemia (1). This review assessed the effectiveness of administering oral dextrose gel to correct hypoglycaemia in newborn infants from birth to discharge and reducing long-term neurodevelopmental impairment. The current evidence shows that oral dextrose gel compared with placebo gel *probably* increases the correction of hypoglycaemic events and may result in a slight reduction in the risk of major neurological disability at 4.5 years corrected age, but the evidence was still uncertain. The fact that oral dextrose gel *probably* corrected hypoglycaemic events and led to higher blood glucose concentrations while reducing maternal-infant separation and improving exclusive breastfeeding after discharge were important indicators of the utility of oral dextrose gel, especially in the absence of evidence of adverse events during the neonatal period.

A cost analysis also reported that treating neonatal hypoglycaemia with oral dextrose gel was likely to result in greater cost savings than a standard approach. Oral dextrose gel is a simple, low-cost, and possibly effective treatment for initial treatment of infants with neonatal hypoglycaemia during the first 48 hours after birth. Available evidence does not support extrapolation to other contexts, or to either extremely or moderately preterm infants. Future studies should examine the use of oral dextrose gel in a variety of settings (resource poor setting) and patient groups (pre-term infants).

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2.3. Continuous glucose monitoring in the management of neonates with persistent hypoglycemia and congenital hyperinsulinism

Win M, Beckett R, Thomson L, Thankamony A, Beardsall K

J Clin Endocrinol Metab. 2022 Jan 1;107(1):e246-e253.

doi: 10.1210/clinem/dgab601. PMID: 34407200.

Brief Summary: This retrospective single center study in neonates with hyperinsulinaemic hypoglycaemia examined the utility of real-time continuous glucose monitoring (CGM). CGM is best placed as an adjunct to routine intermittent blood glucose monitoring, providing information on glucose trends during normoglycemia rather than point accuracy.

Hyperinsulinaemic hyperinsulinism (hyperinsulinism) leads to severe and persistent hypoglycemia. Maintaining normoglycemia in babies with hyperinsulinism can be extremely challenging. Current management strategies involve doing frequent (sometimes hourly) blood sampling, typically heal pricks, in order to detect and treat hypoglycemia. Hourly blood glucose sampling can be stressful for both infants and staff and thus having another method of monitoring glucose levels will be useful.

In this single-center retrospective study the authors assessed the utility of using real-time continuous glucose monitoring (GCM) over a 4-year period in babies with persistent hypoglycemia (these included patients with IUGR and congenital hyperinsulinism babies) concurrent with blood glucose measurements. The study demonstrated that there were marked fluctuations in the CGM readings which made clinical management and prevention of hypoglycaemia challenging when using intermittent blood glucose values. There were high numbers of false-positive CGM readings with limitations in point accuracy.

A previous study also demonstrated high number of false positive hypoglycaemic readings when using GCM in babies with hyperinsulinism (1). Thus, CGM is best placed to act as an adjunct on glucose trends and the timely need for intermittent blood glucose monitoring during normoglycemia rather than point accuracy. The use of CGM to provide reassurance during periods of normoglycemia could potentially limit the need for such frequent blood sampling. The real-time data providing continuous trends that would highlight falling glucose levels and alert the clinician to the need for blood glucose measurement rather than levels simply being taken hourly as part of routine care.

This study also highlighted the need for NICU staff to be trained in using CGM technology which will be critical to using CGM alongside blood glucose monitoring. Newer CGM devices have improved accuracy and no longer require calibration with blood glucose levels but further developments in the technology to optimize use in the newborn would be beneficial.

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2.4. Continuous glucose monitoring for the prevention of morbidity and mortality in preterm infants

Galderisi A, Trevisanuto D, Russo C, Hall R, Bruschettini M

Cochrane Database Syst Rev. 2021 Dec 21;12(12):CD013309.
doi: 10.1002/14651858.CD013309.pub3. PMID: 34931697.

Brief Summary: This systematic review assessed the evidence for continuous glucose monitoring (CGM) to prevent morbidity and mortality in preterm infants. There was uncertainty about the safety of CGM and the available management algorithms, and many morbidities remain unreported in this patient group.

Preterm infants are susceptible to hyperglycaemia and hypoglycaemia, which may lead to adverse neurodevelopment. The hypoglycaemia may be due to a consequence of immature gluconeogenesis and ketogenesis, and to hyperglycaemia due to impaired insulin response to glucose variations during the first days of life (1). The use of CGM devices might help in keeping glucose levels in the normal range, and reduce the need for blood sampling. However, the use of CGM might be associated with harms in the preterm infant.

Based on four identified studies, this review found insufficient evidence to determine if CGM impacts on preterm infant mortality or morbidities. There was uncertainty about the safety of CGM and the available management algorithms, and many morbidities remain unreported. Preterm infants at risk of hypoglycaemia or hyperglycaemia were enrolled in all four included studies. No studies had been conducted in preterm infants with proven hypoglycaemia or hyperglycaemia. Long-term outcomes were not reported. Hence, the effectiveness of CGM on this outcome remains very uncertain.

Clinical trials are required to determine the most effective CGM and glycaemic management regimens in preterm infants before larger studies can be performed to assess the effectiveness of CGM for reducing mortality, morbidity, and long-term neurodevelopmental impairments.

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2.5. PNC2 (SLC25A36) deficiency associated with the hyperinsulinism/hyperammonemia syndrome

Shahrour MA, Lasorsa FM, Porcelli V, Dweikat I, Di Noia MA, Gur M, Agostino G, Shaag A, Rinaldi T, Gasparre G, Guerra F, Castegna A, Todisco S, Abu-Libdeh B, Elpeleg O, Palmieri L

J Clin Endocrinol Metab. 2021 19;107(5):1346-1356.
doi: 10.1210/clinem/dgab932. PMID: 34971397.

Brief Summary: This is a case report of a potentially new genetic disorder that causes hyperinsulinaemic hypoglycemia, protein sensitivity and high serum ammonia level (Hyperinsulinism/hyperammonemia syndrome (HI/HA) syndrome). Mutations in the solute the carrier family 25, member 36 (SLC25A36) may be a novel cause of HI/HA syndrome but more patients need to be identified.

Hyperinsulinism/hyperammonemia syndrome (HI/HA) is an autosomal dominant form of hyperinsulinism due to gain of function mutations in the GLUD1 coding for the mitochondrial enzyme glutamate dehydrogenase (GDH) (1). This is classically associated with protein sensitivity and an elevated serum ammonia level. Rarely there are patients with protein sensitivity and an elevated serum ammonia level but with no mutations in the *GLUD1* gene, suggesting potentially other genetic mechanisms.

This paper describes 2 siblings who have biochemical findings of hyperinsulinism and high serum ammonia levels but no mutations in *GLUD1*. Instead these patients were found to have a homozygous splice site variant in solute the carrier family 25, member 36 (*SLC25A36*), encoding the pyrimidine nucleotide carrier 2 (PNC2), a mitochondrial nucleotide carrier that transports pyrimidine as well as guanine nucleotides across the inner mitochondrial membrane. The authors were able to show that PNC2 impairment likely leads to a reduced mitochondrial GTP content, hence limiting GDH inhibition and hyperactivating insulin secretion pathway.

PNC2 seems to be a new player in the regulation of insulin secretion (2). PNC2 catalyzes the transport of guanosine phosphates (GNPs) in exchange for other nucleotides across the inner mitochondrial membrane, thus feeding the mitochondrial GTP pool. In glucose-stimulated pancreatic beta-cells, a defect in PNC2 may reduce mitochondrial GTP leading to an increase of glutamate oxidative deamination by glutamic dehydrogenase (GDH) and, consequently, a stimulation of TCA cycle activity and oxidative phosphorylation. These are interesting observations and it will be important to find more cases of patients with mutations in *SLC25A36* and further understand the complex mechanisms of how GLUD1 is regulated.

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2.6. The bihormonal bionic pancreas improves glycemic control in individuals with hyperinsulinism and postpancreatectomy diabetes: a pilot study

Rayannavar A, Mitteer LM, Balliro CA, El-Khatib FH, Lord KL, Hawkes CP, Ballester LS, Damiano ER, Russell SJ, De León DD
Diabetes Care. 2021 Nov;44(11):2582-2585.
doi: 10.2337/dc21-0416. PMID: 34518377.

Brief Summary: This pilot study, in patients with post-pancreatectomy diabetes due to congenital hyperinsulinism, assessed pump-delivered insulin and glucagon (Bihormonal Bionic Pancreas, BHBP) to regulate blood glucose levels. BHBP controlled post-prandial glucose levels better than a conventional insulin pump but further studies are needed.

Patients with diffuse congenital hyperinsulinism may require a near-total pancreatectomy if they are unresponsive to medical treatment. The near-total pancreatectomy eventually leads to post-pancreatectomy diabetes (PPD). This type of PPD is associated with insulin and glucagon deficiency. The bi-hormonal bionic pancreas (BHBP, a type of pump) has been shown to improve glycemic control and to reduce the frequency of hypoglycemia in individuals with type 1 diabetes by autonomously administering insulin and glucagon based on plasma glucose levels detected via continuous glucose monitoring (CGM) system (1).

This open-label, randomized crossover pilot study compared current standard diabetes care (using the standard insulin pump) with the BHBP to reduce the mean glucose concentration and the fraction of time with glucose concentrations <3.3 mmol/L in patients with PPD. Although the authors conclude that the use of the BHBP may be better suited for post-prandial glycaemia in individuals with congenital hyperinsulinism than current conventional insulin pump therapy, there are several limitations.

The key limitation is that the study only involved 10 patients and this small sample size had limited ability to demonstrate statistically significant differences in the outcomes. There were no significant differences in the main outcomes between the standard care and the BHBP. Larger and longer studies using the newer BHBP device will need to be undertaken in this population to establish the long-term benefit and risks of the BHBP.

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2.7. Stem cell based models in congenital hyperinsulinism - perspective on practicalities and possibilities

Lithovius V, Otonkoski T

Front Endocrinol (Lausanne). 2022 Feb 18;13:837450.
doi: 10.3389/fendo.2022.837450. PMID: 35250887.

Brief Summary: This paper discusses the use of Pluripotent Stem Cell (PSC)-derived pancreatic islets (SC-islets) for studying the basic biology, molecular mechanisms and therapeutic potentials of patients with congenital hyperinsulinism (CHI). The availability of CHI patient islets opens new avenues for research and development of new treatments.

PSCs represent the epiblast cells of the early embryo, capable of differentiation to any cell type in the human body. Tissue differentiated from PSCs holds enormous promise in regenerative medicine to replace or repair a damaged or degenerated organ. PSCs can also serve as a powerful research tool by allowing limitless generation of difficult-to-procure tissue and would thus serve as an attractive solution for preclinical study of CHI.

PSCs can be derived from two main sources 1) from preimplantation embryos (embryonic stem cells, ESCs) and 2) from somatic cells that have been reprogrammed back to pluripotent state by overexpression of key genes (induced pluripotent stem cells, iPSCs). iPSCs reprogrammed from a patient sample carry the disease-causing mutations of that individual and should thus phenocopy the disease, such as CHI, when differentiated. A similarly differentiated healthy iPSC line would serve as a non-isogenic control for this type of approach.

Stem cell derived islets represent a powerful tool for modeling diseases of the pancreatic beta cell, due to the potential to produce them in limitless quantities with high consistency and with high disease phenotype fidelity. In the case of CHI, the SC islets can be harnessed to discover novel anti-hypoglycemic medications, to study molecular mechanisms of newly discovered CHI genes and to study the basic biology of a hyperactive beta cell.

Thus, the modeling of CHI with SC-islets will serve as a critical next step required for the development of specific and efficient anti-hypoglycemic drugs. The availability of CHI patient islets presents an additional challenge due to the rarity of the disease. The limited tissue availability challenges any study that requires large amounts of tissue, such as screening for novel pharmacotherapeutics.

2.8. CRISPR/Cas9 *ADCY7* knockout stimulates the insulin secretion pathway leading to excessive insulin secretion

Alhaidan Y, Christesen HT, Lundberg E, Balwi MAA, Brusgaard K

Front Endocrinol (Lausanne). 2021 Jun 11;12:657873.
doi: 10.3389/fendo.2021.657873. PMID: 34177802.

Brief Summary: This is a single case report describing a possible new candidate gene (*ADCY7*) for congenital hyperinsulinism. Mutations in the *ADCY7* gene may be a new cause of congenital hyperinsulinism but more cases are needed and the molecular mechanisms studied in more detail.

A 4-month-old boy presented with hyperinsulinaemic hypoglycemia that was medically responsive to treatment with diazoxide. Routine genetic testing did not detect any mutations in the known genes for hyperinsulinaemic hypoglycemia. However, whole exome sequencing identified a variant in the adenylyl cyclase 7 (*ADCY7*) gene p.(Asp439Glu) and p.(Gly1045Arg). *ADCY7* encodes a membrane-bound adenylate cyclase that converts ATP to cyclic AMP and pyrophosphate (1). Using a RIN-m cell line and transfection studies the authors showed increased insulin secretion when *ADCY7* was knocked down.

However, the underlying molecular mechanism of increased insulin secretion due to loss of function in the *ADCY7* gene is unclear. The complexity of the relationship between *ADCY7* and insulin secretion was at least in part explained by the changes in glucose sensing and glucose uptake in beta cells, which regulates insulin secretion via the glucose stimulated-insulin secretion pathway.

The role of *ADCY7* in glucose induced insulin secretion needs to be further tested in other cell lines, such as the human pancreatic islets, EndoC-bH1 and rat pancreatic cells, BRIN-BD11 and INS-1 and more patients need to be identified with mutations in the *ADCY7* gene before this is considered as a novel candidate gene for congenital forms of hyperinsulinism.

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Neonatal Diabetes Mellitus

2.9. SGLT2 inhibitors therapy protects glucotoxicity-induced β-cell failure in a mouse model of human KATP-induced diabetes through mitigation of oxidative and ER stress

Shyr ZA, Yan Z, Ustione A, Egan EM, Remedi MS

PLoS One. 2022 Feb 18;17(2):e0258054.
doi: 10.1371/journal.pone.0258054. PMID: 35180212.

Brief Summary: This mouse model of diabetes describes how early administration of sodium glucose transporter 2 (SGLT2) inhibitors can protect pancreatic beta-cells from glucotoxicity damage. Early use of SGLT2 inhibitors can revert/prevent beta-cell failure in mice with diabetes due to KATP channel defects.

Chronic hyperglycemia in type 1 and type 2 diabetes induces beta-cell membrane hyperexcitability, persistently elevated intracellular calcium concentrations and insulin hypersecretion as well as oxidative and endoplasmic reticulum (ER) stress. This all leads to beta-cell exhaustion, loss of beta cell function and mass. Loss of beta-cell mass also occurs in KATP-gain-of-function (KATP-GOF) mouse model of human neonatal diabetes mellitus (NDM) but in this case there is no insulin hypersecretion. Little is known about the underlying mechanisms and temporal progression of loss of functional beta-cell mass in monogenic diabetes, in the absence of compensatory increase in insulin secretion.

SGLT2 inhibitors are a new class of antidiabetic drugs that inhibit glucose reabsorption in the kidneys and increase glucose excretion in the urine. Because their mechanism of action is independent of insulin secretion or action, SGLT2 inhibitors can be used in combination with other therapies. Individuals with Type 2 diabetes treated with SGLT2 inhibitors demonstrate improved glycemic control, increased glucose- and incretin-stimulated insulin secretion and enhanced insulin sensitivity as well as reduced blood pressure, decreased plasma lipids and reduced risk for cardiovascular events (1).

Although improved beta-cell function by SGLT2 inhibitors has been suggested in humans and rodents, the underlying mechanisms and timeframe of this effect remain unclear, with most studies performed in the setting of obesity and type 2 diabetes (2). KATP-GOF mice model show an unexpected loss of insulin content, decreased proinsulin processing and increased proinsulin at 2-weeks of diabetes accompanied marked increase in beta-cell oxidative and ER stress, without changes in islet cell identity. Administration of the SGLT2 inhibitor dapagliflozin restored insulin content, decreased proinsulin: insulin ratio and reduced oxidative and ER stress but this was only effective if given before 40 days from the onset of the diabetes (when loss of beta-cell mass and identity had already occurred).

Thus, it is the hyperglycemia per se, and not insulin hypersecretion that drives beta-cell failure in diabetes and recovery of beta-cell function by SGLT2 inhibitors is potentially through reduction of oxidative and ER stress. SGLT2 inhibitors revert/prevent beta-cell failure when used in early stages of diabetes, but not when loss of beta-cell mass/identity already occurred. This has important clinical implications for the early use of SGLT2 inhibitor therapy for diabetes mellitus.

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2.10. Genetic reduction of glucose metabolism preserves functional β-cell mass in KATP-induced neonatal diabetes

Yan Z, Fortunato M, Shyr ZA, Clark AL, Fuess M, Nichols CG, Remedi MS

Diabetes. 2022 1;71(6):1233-1245.
doi: 10.2337/db21-0992. PMID: 35294000.

Brief Summary: This mouse model of diabetes tested the hypothesis that reducing the metabolic flux (rate of metabolism) in the beta cell can prevent beta-cell failure and preserve beta-cell mass. Reducing glucose metabolism may be a mechanism for preventing glucotoxicity-induced loss of functional beta-cell mass in diabetes.

Beta-cell exhaustion, loss of mass and function are thought to occur due to insulin hypersecretion in both type 1 and type 2 diabetes. However, beta-cell exhaustion, loss of mass and function also occur in a monogenic form of neonatal diabetes mellitus to gain of function mutations in the KATP channel where there is no insulin hypersecretion (1). To test the hypothesis that it is the beta-cell hyperglycemia induced metabolism that leads to beta-cell failure, the authors crossed KATP-GOF (Gain of function)–induced neonatal diabetic mice with beta-cell–specific GCK haplo-insufficient mice (GCK1/, Glucokinase 1)). GCK is a key enzyme which allows the entry of glucose into the beta-cells.

The beta-cell specific GCK haplo-insufficient mice (GCK1/) allows less glucose to enter the beta-cell and thus reduces the metabolic flux in the beta-cell. The double-mutant KATP-GOF/GCK1/ mice had slower progression of diabetes development and the rate of metabolism was reduced in the beta-cell. In addition, the insulin content of the beta-cells was preserved as was the beta-cell mass and identity. The double mutant mice also showed increased insulin sensitivity and restoration of body weight and liver and brown/white adipose tissue mass and function and normalization of physical activity and metabolic efficiency thus suggesting that the other tissues are also impacted by the reduction in the metabolic flux in the beta-cell. Therefore, a reduction in the beta-cell glucose metabolism seems to be protective for insulin preservation and beta-cell identity.

Thus, paradoxically, reducing glucose metabolism may be a mechanism to prevent glucotoxicity-induced loss of functional beta-cell mass in diabetes and to maintain adipose tissue and liver function. However, these observations need to be tested in human pancreatic beta-cells which have many differences to rodents.

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2.11. Cognitive deficits and impaired hippocampal long-term potentiation in KATP-induced DEND syndrome

Yahil S, Wozniak DF, Yan Z, Mennerick S, Remedi MS

Proc Natl Acad Sci U S A. 2021 Nov 9;118(45):e2109721118.

doi: 10.1073/pnas.2109721118. PMID: 34732576.

Brief Summary: In a mouse model of KATP neonatal diabetes mellitus, this study examined the mechanisms of cognitive deficits and development delay observed in some patients with this form of diabetes. The cognitive deficits and development delay appeared to be independent of the diabetes per se.

Gain of function mutations in the KATP channel genes are the most common cause of neonatal diabetes mellitus (NDM) in some parts of the world (1). Some patients with NDM due to gain of function in the KATP channel genes also have epilepsy, cognitive defects and developmental delay (DEND syndrome). However, the underlying mechanisms are not clear.

These authors phenotyped mice with gain of function mutations in the KATP channels either specifically in the hippocampus or pan-neuronally and compared these mice with pancreatic KATP channel knockout mice. The pan-neuronal mice exhibited some similar cognitive features as found in DEND syndrome. The hippocampal expression of KATP gain of function was associated with cognitive but not sensorimotor deficits and associated with learning and memory defects. Hippocampal neurons from pan-neuronal and hippocampal specific KATP channel mice showed sensitivity to KATP channel openers and inhibitors. Learning and memory deficits were not improved by sulfonylurea therapy. Interestingly, mice with KATP gain of function in the pancreatic beta-cell had diabetes but no cognitive deficits.

These findings indicate that KATP-GOF (gain of function) channels in the hippocampal region play an important role in learning and memory deficits and that the cognitive deficits in DEND syndrome result from neuronal KATP-GOF expression rather than from diabetes per se. The inefficacy of sulfonylureas to improve cognitive deficits in mice agrees with the limited neurological improvements observed with treatment in human DEND.

These studies have important clinical implications, pointing to potential mechanisms underlying cognitive deficits of KATP-induced DEND syndrome and indicating the need for novel drugs to treat neurological features arising from KATP-GOF mutations while also providing a platform to study other brain abnormalities induced by ion channel dysfunction.

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2.12. Developmental defects and impaired network excitability in a cerebral organoid model of KCNJ11 p.V59M-related neonatal diabetes

Dalgin G, Tryba AK, Cohen AP, Park SY, Philipson LH, Greeley SAW, Garcia AJ 3rd

Sci Rep. 2021 Nov 3;11(1):21590.

doi: 10.1038/s41598-021-00939-7. PMID: 34732776.

Brief Summary: This study describes the molecular and electrophysiological analysis of the cerebral network generated from cerebral organoids from human induced pluripotent stem cells (hiPSCs) on a patient with neonatal diabetes mellitus due to a *KCNJ11* mutation.

Some patients with permanent neonatal diabetes mellitus (PNDM) due to activating *KCNJ11* (KATP channel subunit) gene mutations have neurological deficits such as severe learning disorders, cognitive disorders such as autism spectrum like disorder and epilepsy. KATP channels also play a role in coupling neuronal metabolism to electrical excitability and neurotransmitter release and this influences the development of neuronal circuits during neurogenesis. Advances in human induced pluripotent stem cell and 3D cell culture technology have made it possible to generate brain tissue called cerebral organoids, in vitro (1). Cerebral organoid technology provides a good model for understanding neural differentiation as well as the effects of genetic variation on gene expression of brain development and disease.

The mechanisms of the learning disorders and cognitive disorders, such as autism spectrum-like disorder and epilepsy due to activating *KCNJ11* mutations is/are not clear. So, in order to understand the electrophysiological role of the *KNCJ11* mutation (V59M) in the cerebral network, the authors created first an iPSC line from a patient with PNDM. They then converted the iPSC into cerebral organoids and studied the electrophysiology of the neuronal networks. The mutant organoid showed impaired neurodevelopment, and impaired neuronal differentiation with defects in the laminar organization of the neocortex development. The decreased neurogenesis resulted in defective neural circuit formation and activity with failure to migrate and differentiate normally. Interestingly pharmacological treatment with tolbutamide (sulphonylurea which blocks the KATP channel) partially rescued the molecular defects caused by hyperpolarization of the cell membrane.

Overall, these findings suggest that the mutant KCNJ11 channel activity dysregulates neuronal circuit formation and neuronal network excitability in human cerebral samples. The study was able to disentangle the confounding effects of neonatal diabetes mellitus from the direct influence of the V59M mutation on neural precursors and neurons. This study provides the first evidence that a mutant KCNJ11 channel directly causes neurological deficits in patient hiPSC derived brain tissue.

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2.13. An induced pluripotent stem cell line derived from a patient with neonatal diabetes and Fanconi-Bickel syndrome caused by a homozygous mutation in the SLC2A2 gene

Elsayed AK, Al-Khawaga S, Hussain K, Abdelalim EM

Stem Cell Res. 2021 Jul;54:102433.
doi: 10.1016/j.scr.2021.102433. PMID: 34171785.

Brief Summary: This study generated induced pluripotent stem cells (iPSCs) from a patient with permanent neonatal diabetes mellitus (PNDM) due to Fanconi-Bickel syndrome. This iPSC line provides a novel human cell model to understand the pathophysiology of FBS and diabetes mellitus and for the potential of developing and testing new pharmacological treatments.

Fanconi-Bickel syndrome (FBS) is a rare disorder caused by homozygous mutations in the *SLC2A2* gene. This gene encodes for the GLUT2 glucose transporter which is expressed in the pancreatic beta-cells, liver, central nervous system and the kidney. Some patients with FBS may present with permanent neonatal diabetes mellites (PNDM). GLUT2 seems to have an important role in beta-cell physiology but the underlying mechanisms involved are not known (1). Thus, having a cell line which has a mutated GLUT2 would be very useful to study the mechanisms of GLUT2 and pancreatic beta-cell physiology. Using the patient's peripheral mononuclear cells (PBMCs), the authors generated iPSCs from a patient with a homozygous nonsense mutation in the *SLC2A2* gene and PNDM.

The PBMCs were reprogramed into a pluripotent state using the non-integrating Sendai virus vector expressing OCT4, SOX2, c-MYC, and KLF4. The mutation (c.901C T) in the *SLC2A2* gene was confirmed in the generated iPSC line using sanger sequencing. The generated cell line displayed an identical morphology of the human embryonic stem cell colonies and expressed the key pluripotency markers, including OCT4, SSEA4, SOX2, TRA-1–60, NANOG, TRA81, c-MYC, KLF4, REX1 and TERT as examined by immunocytochemistry, RT-PCR, and flow cytometry analyses.

This iPSC line provides a novel human cell model to understand the pathophysiology of FBS and diabetes mellitus associated with *SLC2A2* defects and for the potential of testing new pharmacological treatments.

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2.14. Mutations and variants of *ONECUT1* in diabetes

Philippi A, Heller S, Costa IG, Senée V, Breunig M, Li Z, Kwon G, Russell R, Illing A, Lin Q, Hohwieler M, Degavre A, Zalloua P, Liebau S, Schuster M, Krumm J, Zhang X, Geusz R, Benthuysen JR, Wang A, Chiou J, Gaulton K, Neubauer H, Simon E, Klein T, Wagner M, Nair G, Besse C, Dandine-Roulland C, Olaso R, Deleuze JF, Kuster B, Hebrok M, Seufferlein T, Sander M, Boehm BO, Oswald F, Nicolino M, Julier C, Kleger A

Nat Med. 2021 Nov;27(11):1928-1940.
doi: 10.1038/s41591-021-01502-7. PMID: 34663987.

Brief Summary: This clinical study characterised the spectrum of novel diabetes phenotypes due to mutations in the Transcription factor One Cut Homeobox 1 (*ONECUT1*)/hepatocyte nuclear factor 6 (HNF6). The study uncovers novel forms of diabetes mellitus due to mutations in *ONECUT1*.

The One Cut Homeobox 1 (*ONECUT1*) transcription factor promotes differentiation of endocrine and duct cells of the pancreas (1). It is required for timely specification of the pancreas, controls expression of the ngn3 gene and acts upstream of Pdx-1 in the specification cascade in pancreatic development. In addition, it is essential for differentiation and morphogenesis of the biliary tract.

The authors identified homozygous loss of function mutations in the *ONECUT1* transcription factor in 2 unrelated patients who had a syndromic form of permanent neonatal diabetes mellitus (with pancreatic hypoplasia and gallbladder agenesis/hypoplasia). Then they studied the heterozygous carriers of the mutation and noted that these carriers had a distinctive subgroup of diabetes mellitus with early-onset, non-autoimmune (antibody negative) diabetes which was responsive to treatment with oral medications such as oral sulphonylureas (a phenotype resembling Maturity Onset Diabetes of the Young, MODY). In addition, common regulatory *ONECUT1* variants were associated with multifactorial type 2 diabetes.

To understand the molecular basis of the pancreatic hypoplasia and the diabetes mellitus, the authors generated induced pluripotent stem cells from the patients. Functional analysis of these induced pluripotent stem cells showed that the *ONECUT1* mutations impair pancreatic progenitor formation and the subsequent endocrine program. *ONECUT1* mutations led to altered binding of the transcription factor and affected the enhancer activity of other key pancreatic transcription factors such as NKX2.2/NKX6.1 in pancreatic progenitor cells.

This study has uncovered novel forms of diabetes mellitus due to homozygous and heterozygous mutations in the *ONECUT1* transcription factor. This transcription factor has a pivotal role in the developmental and function of the pancreas and defects lead to broad spectrum of diabetes phenotypes depending in the type of mutation. It controls a transcriptional and epigenetic machinery regulating endocrine development.

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Gestational Diabetes Mellitus: Neonatal and Long-term Consequences

2.15. Migration, gestational diabetes, and adverse pregnancy outcomes: a nationwide study of singleton deliveries in Denmark

Kragelund Nielsen K, Andersen GS, Damm P, Nybo Adersen AM

J Clin Endocrinol Metab. 2021 Nov 19;106(12):e5075-e5087.
doi: 10.1210/clinem/dgab528. PMID: 34272865.

Brief Summary: This national birth registry study in Denmark collected data on gestational diabetes and adverse pregnancy outcomes in relation to country of origin of the mother. Country of origin and the number of births were associated with adverse effects from gestational diabetes.

There is a global variation in the prevalence of gestational diabetes mellitus (GDM) with the risk being particularly high in Asian women (1). Maternal country of origin may affect the outcomes of GDM such as pre-eclampsia, planned caesarean section, emergency caesarean section, preterm delivery, large for gestational age (LGA) and small for gestational age (SGA). For example, among women giving birth in Denmark, women born in Pakistan, India, Morocco, and Sri Lanka have 3- to 5-fold higher odds of GDM compared with native Danish-born women (2).

So, to address the question if the maternal country of origin has any adverse effects on GDM outcome, the authors used the Denmark nationwide singleton birth database to collect information on maternal origin, GDM and pregnancy outcomes. They found that GDM increased the odds risk of adverse pregnancy outcomes. More specifically, country of origin modified the effect of GDM on pre-eclampsia, LGA, and SGA in singleton deliveries. However, there was no effect of GDM on planned cesarean section, emergency cesarean section, and preterm delivery. Different immigrant groups had higher odds of different GDM-associated adverse pregnancy outcomes, and there were differences in GDM-associated adverse pregnancy outcomes between countries of origin often grouped together.

For example, GDM increased the risk of pre-eclampsia among women from Denmark, Lebanon and Morocco, and GDM was associated with increased risk of LGA among women from most countries, particularly women from Sri Lanka. The odds of having a neonate with SGA was increased in women with GDM who came from India, Lebanon, Pakistan, Iraq, and Somalia. Thus, this study highlights the importance of increased awareness to both immigrant background and GDM status in the clinical assessment of pregnant women.

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2.16. Maternal glycemia during pregnancy and early offspring development: a prospective birth cohort study

Wang P, Xie J, Jiao XC, Ma SS, Liu Y, Yin WJ, Tao RX, Hu HL, Zhang Y, Chen XX, Tao FB, Zhu P

J Clin Endocrinol Metab. 2021 Jul 13;106(8):2279-2290.
doi: 10.1210/clinem/dgab331. PMID: 33982055.

Brief Summary: This prospective birth cohort study assessed the impact of maternal glycaemia on infant neurodevelopment at 12 months of age. Maternal glucose levels during pregnancy were associated with infant neurodevelopmental outcomes.

High maternal blood glucose levels may have a negative effect on fetal neurodevelopment outcome, although the evidence is unclear. To study this in more detail, the authors undertook a prospective birth cohort study of women with GDM (mother child-pairs) and assessed neurodevelopment outcome of the infants at 12 months of age using a questionnaire.

The study showed that maternal GDM was associated with developmental delay in the offspring. Infants born to mothers with GDM were more likely to fail the communication domain than those of mothers without GDM. After adjusting for confounders (such as maternal age, maternal education, maternal life-style and husbands' education) there remained a positive association of maternal GDM and problems in the communication domain. The higher the maternal blood glucose levels, the greater was the difference in the communication domain and personal social domain. For each standard deviation higher maternal glucose the risk of failing the communication domain increased from 32% to 70%.

The relationships between maternal glucose and risks of poorer neurodevelopment were continuous without evidence of any threshold effect. Cord blood C-peptide levels > 90th centile were associated with higher risk of failing the communication domain. Potential mechanisms include an adverse effect of fetal C-peptide on

neurodevelopment through the nitric oxide signaling pathway, hypoxia, oxidative stress, or differences in gut microbiota between GDM and non-GDM mothers (1, 2).

This is the first prospective birth cohort study to show a positive linear association of maternal glucose levels with poor developmental outcomes. These findings are important and suggest that earlier screening and management of the maternal glycaemia might prevent or delay developmental changes in infants.

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2.17. Persistence of risk for type 2 diabetes after gestational diabetes mellitus

Diaz-Santana MV, O'Brien KM, Park YM, Sandler DP, Weinberg CR

Diabetes Care. 2022 Apr 1;45(4):864-870.
doi: 10.2337/dc21-1430. PMID: 35104325.

Brief Summary: This study followed up a nationwide cohort of pregnant women (with and without gestational diabetes, GDM) and assessed their risk of developing type 2 diabetes mellitus up to 10 years later. The risk of developing type 2 diabetes increased with the number of GDM pregnancies.

About 6% of pregnancies are affected by GDM, and women with GDM have increased risk of later type 2 diabetes (T2D). A meta-analysis estimated that the risk for later T2D among women with GDM is 10-fold higher compared with non-GDM women (1). However, it is unclear if this risk of T2D depends on the number of affected pregnancies or for how long the risk persists.

These authors followed up a nationwide cohort of women over a 10-year period after pregnancy and assessed how the risk of T2D varies with BMI and the cumulative number of GDM pregnancies, and how the age-specific relative risk of T2D changes over time. A history of GDM predicted greatly increased rates of developing T2D. The relative risk increased substantially with each additional GDM pregnancy. Women with > 3 GDM pregnancies who were within 6–15 years of their last GDM pregnancy had ~ 7-fold higher risk of T2D compared with those without GDM. In addition, although the age-adjusted relative risk of T2D declined over time since the most recent GDM diagnosis, it remained elevated for >35 years.

How might GDM increase the risk of later T2D? The precise reasons for this are unclear, but GDM leads to pancreatic beta-cell dysfunction in women with pre-existing insulin resistance and this might be progressive (2). In addition, GDM itself may have deleterious effects on beta-cell function.

These observations have important clinical implications as women with GDM should be screened regularly for T2D, especially those with multiple GDM pregnancies. Additionally, women with a history of GDM who also have a BMI in the overweight or obese category, should be screened regularly for T2D, even late in life.

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2.18. Novel epigenetic link between gestational diabetes mellitus and macrosomia

Joyce BT, Liu H, Wang L, Wang J, Zheng Y, Nannini D, Drong A, Shiau S, Li W, Leng J, Shen Y, Gao R, Baccarelli A, Hu G, Hou L

Epigenomics. 2021 Aug;13(15):1221-1230.
doi: 10.2217/epi-2021-0096. PMID: 34337972.

Brief Summary: This case cohort study assessed whether epigenetic factors explain the link between gestational diabetes mellitus (GDM) and macrosomia. Epigenetic changes in the *MEST* gene were associated with both GDM and macrosomia.

GDM leads to neonatal macrosomia and in the long-term to the metabolic syndrome. The underlying mechanisms of the macrosomia and metabolic syndrome are unknown but epigenetic changes might be involved. GDM has been associated with placental DNA methylation changes in some epigenome-wide association studies. It remains unclear which genes or pathways are affected, and whether any placental differential gene methylations are correlated to fetal growth or circulating metabolic health biomarkers

The authors selected 8 genes (*IGF1*, *IGF2*, *H19*, *ARHGRF11*, *MEST*, *NR3C1*, *Adiponectin*, and *RETN*) which were known to be involved in GDM and obesogenic pathways and assessed the methylation status of these in GDM mothers and their macrosomic offspring. Epigenetic changes were found mainly in the *MEST* gene (Mesoderm-specific transcript) which is paternally imprinted and highly expressed in fetal and placental tissue and is believed to play an important role in fetal development.

There is now increasing evidence that downstream pathologies of obesity are amplified or even initiated by molecular changes within the white adipose tissue (WAT). Such changes are the result of an excessive expansion of individual white adipocytes and could potentially be ameliorated via an increase in *de novo* adipocyte recruitment (adipogenesis). MEST is a protein with a putative yet unidentified enzymatic function and has previously been shown to correlate with adiposity and adipocyte size in mice. *MEST* expression is upregulated during human adipocyte differentiation, increased in human white adipose tissue in the obese state and significantly correlated with adipocyte volume. Thus, epigenetic changes in *MEST* may lead to macrosomia in mothers with GDM.

Maternal Obesity and Long-term Infant Consequences

2.19. Association of infant diet with subsequent obesity at 2-5 years among children exposed to gestational diabetes: the SWIFT study

Vandyousefi S, Davis JN, Gunderson EP
Diabetologia. 2021 May;64(5):1121-1132.
doi: 10.1007/s00125-020-05379-y. PMID: 33495846.

Brief Summary: This longitudinal mother-infant dyad study assessed the risk of obesity at age 2-5 years in offspring who had been exposed to Gestational diabetes mellitus (GDM), were breastfed for either less or more than six months, and also exposed to sugar-sweetened beverages.

GDM, limited breastfeeding (BF) or exclusive BF (EBF), and other postnatal dietary habits may influence weight gain, obesity and metabolic disease among young children and adolescents. Early introduction of complementary foods, sugar-sweetened beverages (SSB) and unsweetened fruit juice have been associated with obesity in young children. The American Academy of Pediatrics (AAP) recommends breast milk to be the sole source of nutrition during the infant's first 6 months of life and other nutrients introduced after 6 months of age. The AAP also recommends avoiding fruit juice and SSBs during the first 6 months of life because of the high sugar content.

The authors followed up infants of GDM mothers and obtained information about the frequency and duration of BF and exposure to fruit juice and SSB during the first 6 months. The risk of obesity at 2-5 years was higher if the infants had less breast milk and had intake of fruit juice and SSB in the first 6 months of life. Having less breast milk itself was associated with the risk of obesity but the introduction of fruit juice and SSB in the diet increased the odds of developing obesity (independent and joint associations).

The exact mechanism(s) how BF may confer protection from obesity are unclear. One possible theory is it encourages the infant's emerging self-regulation of intake, reducing problematic feeding behaviors on the part of caregivers that interfere with the infant's self-regulation of intake, and providing bioactive factors that regulate

energy intake, energy expenditure, and cellular chemistry. How fruit juice and SSB intake during early infancy causes obesity is also unclear. Sweet taste and maternal feeding practices related to nutritional habits and soothing children with foods and liquid feeding may be associated with higher daily SSB intake and consequent child obesity.

In summary, infant feeding exposures, including sugary beverages, may play a major role in countering fetal life programming, and that modification of early postnatal infant feeding habits may be beneficial in ameliorating the risks to the child from intrauterine exposure to maternal hyperglycaemia.

2.20. Obesity class impacts adverse maternal and neonatal outcomes independent of diabetes

Neal K, Ullah S, Glastras SJ

Front Endocrinol (Lausanne). 2022 Mar 24;13:832678.

doi: 10.3389/fendo.2022.832678. PMID: 35399939.

Brief Summary: This retrospective case note study assessed the impact of different classes of obesity on maternal and neonatal outcomes. Higher classes of maternal obesity were associated with increased rates of caesarean section, GDM and gestational hypertension and pre-eclampsia.

Obesity in pregnancy is a risk factor for adverse effects for the mother, neonate and later in childhood. In pregnancy, maternal obesity increases the risk of gestational diabetes mellitus (GDM), gestational hypertension, pre-eclampsia, instrumental delivery, Caesarean section delivery and stillbirth. Women with obesity are more likely to deliver a neonate with congenital abnormalities, large-for-gestational age (LGA) and respiratory distress syndrome. There are only few studies assessing the perinatal outcome based on the degree of maternal obesity. So, this study examined perinatal outcomes in a cohort of women categorized by degree of obesity (class 1 BMI 30.0 to 34.9 kg/m2, class II BMI 35.0 to 39.9 kg/m2 and class III and above BMI $\geq$40 kg/m2)).

Women with obesity class III had increased rates of caesarean section, GDM and gestational hypertension and pre-eclampsia. Stillbirth incidence was greater in women with higher obesity class. The most striking neonatal risk associated with higher class of obesity was LGA, even after adjustment for confounding variables including diabetes in any form. Women with obesity class III had a 49% increased risk of LGA. There was a significant association between obesity class and pre-eclampsia, such that women with obesity class III had at least a two-fold increased risk of pre-eclampsia compared to women with class I obesity.

The incidence of pre-eclampsia in women with obesity was 3.4% increasing to 6.2% in the women with obesity class III. However, Maternal Obesity and Long-term Infant Consequences was not greater in women with higher obesity class; instead maternal diabetes was the major determinant of Maternal Obesity and Long-term Infant Consequences . Women with class III obesity were more likely to deliver a neonate with a birth defect, however this effect was no longer significant after adjusting for other known risk factors, including diabetes and advanced maternal age.

These observations indicate that work in pre-conception weight management should be considered to reduce the number of women entering pregnancy with higher class of obesity.

Fetal and Neonatal Cortisol Physiology

2.21. Preterm birth and infant diurnal cortisol regulation

Stoye DQ, Boardman JP, Osmond C, Sullivan G, Lamb G, Black GS, Homer NZ, Nelson N, Theodorsson E, Reynolds RM, Mörelius E

Arch Dis Child Fetal Neonatal Ed. 2022 Mar 14:fetalneonatal-2021-323296.

doi: 10.1136/archdischild-2021-323296. Epub ahead of print. PMID: 35288450.

Brief Summary: This study tested the hypothesis that the diurnal cortisol area under the curve (mean daily level) and diurnal cortisol slope (decline across the day) differ between infants born preterm and those born at term. Extremely preterm infants showed an abnormal pattern of cortisol secretion characterised by a flat cortisol slope, and this was most marked after the first 6 months of life.

Infants born with low birth weight have an activated hypothalamic–pituitary–adrenal (HPA) axis in early childhood and this may lead to adverse cardiometabolic and neuropsychiatric phenotypes in later adulthood. Previous studies (1) have shown that the basal cortisol concentration of extremely preterm infants, compared with term infants, change from low to high concentrations between 3 and 8 months of age. This suggests that the HPA axis regulation may differ in preterm infants across infancy.

The authors measured the salivary cortisol levels at three-time points, morning, midday and evening on a monthly basis in three cohort of infants (term, very preterm, 28-32 weeks and extremely preterm infants, <27 weeks) until the age of 1 year. The extremely preterm infants (and not the very preterm) had a flat diurnal cortisol slope (defined as the decline in cortisol across the day) when compared to the corrected gestations for infants born at term. The cortisol slope is the difference in the cortisol level between the morning and the evening and thus a flat slope would indicate minimal difference between the cortisol level in the morning and in the evening. This could indicate a loss of diurnal variation in cortisol secretion. These differences were more profound when assessed during corrected ages 7–12 months. The differences between birth groups were more marked when compared at chronological rather than corrected ages. However, the diurnal cortisol area under the curve (indicating total cortisol secretion) did not differ between the birth groups.

These observations suggest that infants who are born extremely preterm (but not the very preterm) have an abnormal pattern of cortisol secretion most marked after the first 6 months of life, characterised by a flat cortisol slope. A metanalysis in adults showed significant associations between flatter diurnal cortisol slopes and poorer health (2). Flatter diurnal cortisol slopes may both reflect and contribute to stress-related dysregulation of central and peripheral circadian mechanisms, with corresponding downstream effects on multiple aspects of biology, behavior, and health. Larger studies will be needed in the extremely preterm infants to replicate these findings.

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Miscellaneous

2.22. Large birth size, infancy growth pattern, insulin resistance and β-cell function

Huang R, Dong Y, Nuyt AM, Levy E, Wei SQ, Julien P, Fraser WD, Luo ZC

Eur J Endocrinol. 2021 May 24;185(1):77-85.
doi: 10.1530/EJE-20-1332. PMID: 33914700.

Brief Summary: This case control study assessed beta-cell function and glucose metabolism in large for gestational age (LGA) infants in relation to their weight and growth parameters at 2 years of age. In LGA infants, changes in growth parameters were associated with changes in insulin resistance and beta-cell dysfunction.

It is known that high birth weight and LGA are associated with higher risk of developing type 2 diabetes in adulthood. Being born LGA is associated with insulin resistance at or shortly after birth and during childhood or adolescence, and also with lower beta-cell function at birth. However, it is unknown whether LGA is associated with insulin resistance and beta-cell dysfunction in infancy (0–2 years, a critical period of rapid postnatal growth and development). So, in this study the authors hypothesized that LGA may be associated with insulin resistance

or lower beta-cell function in infancy, and infancy growth patterns (changes in length) may affect insulin resistance or beta-cell function.

This study showed that LGA was not associated with insulin resistance or beta-cell dysfunction in infancy but there were associations with infant growth parameters. Both accelerated and decelerated growth in length during infancy were associated with beta-cell dysfunction. Accelerated growth in length during mid-infancy was associated with higher HOMA-IR, whereas decelerated growth in length during late infancy was associated with lower HOMA-IR. Accelerated and decelerated changes in weight during infancy were not associated with any significant changes in insulin resistance as assessed by HOMA-IR.

So how does decelerated or accelerated growth in length during infancy affect beta-cell function? The answer is not completely clear but growth in length reflects bone growth and there may be a link between bone and pancreas. Bone marrow cells co-transplanted with islets improves islet vascularization and function in mice (1). Osteoprotegerin, a critical factor involved in bone metabolism, promotes islet cell proliferation in rats (2). Since nutrition is the main driver of decelerated or accelerated growth in infants, both insufficient and excessive nutrition during early and late infancy may be harmful for cell function. However, these observations require confirmation in larger cohort studies.

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2.23. Effect of maternal metformin treatment in pregnancy on neonatal metabolism: evidence from newborn metabolic screening

Estrella J, Wiley V, Simmons D, Hng TM, McLean M
Diabetes Care. 2021 Nov;44(11):2536-2541.
doi: 10.2337/dc21-0327. PMID: 34475030.

Brief Summary: This retrospective case control study investigated changes in the metabolic profile in newborn screening of infants born to mothers who had been treated with metformin. Treatment with metformin during pregnancy was associated with subtle changes in metabolites in the newborn screening.

Metformin has clear benefits in relation to glucose metabolism and diabetes-related complications. The mechanisms underlying these benefits are complex and still not fully understood. It reduces hepatic glucose production, yet not all of its effects can be explained by this mechanism and there is increasing evidence of a key role for the gut (1). Metformin has been shown to act via both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms; by inhibition of mitochondrial respiration but also perhaps by inhibition of mitochondrial glycerophosphate dehydrogenase. Despite its multiple effects on metabolic networks, metformin use seems safe during pregnancy. There are limited data on the effects of metformin on neonatal metabolism

The authors hypothesized that maternal hyperglycaemia and or metformin exposure at different stages of pregnancy might produce an analyte pattern reflective of the growing needs of the embryo and fetal cellular differentiation and maturation. So, they analyzed separately the results of infants exposed from the first, second, or third trimester of pregnancy (with exposure continuing until delivery) to hyperglycaemia or metformin use using the newborn screening.

Exposure to hyperglycaemia during the different stages of pregnancy led to subtle (but significant) changes in some of the metabolites in the newborn screening. These changes were mostly in the acyl-carnitines (short, medium and long) and lower levels of the amino acid leucine. Maternal metformin led to higher levels (but within the normal range) of some specific acyl-carnitines, namely butryl-carnitine (C4), isovalerly-carnitine (C5) and glutaryl-carnitine (C5D). These changes varied according to the gestational age at which metformin was started and hence with duration of exposure. The most consistent changes associated with metformin exposure were in isovalerly-carnitine (C5) concentrations, a short-chain fatty acid esterified to carnitine. These

acyl-carnitines are usually formed by catabolism of the branched chain amino acids leucine and valine and can easily cross the mitochondrial membrane without the need for transporters, conserving cellular energy.

The observed metabolite profile could represent metformin effects on metabolic networks to preferentially express these energy substrates, which may be advantageous in circumstances of insulin resistance or catabolic stress. The observed changes are subtle and do not present any obvious harm to the fetus or newborn.

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3. Thyroid

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Introduction

Over the last year, the most important advances in pediatric thyroidology were made in the field of pediatric differentiated thyroid cancer. Two articles provided evidence for the importance of molecular diagnostics for outcome prediction and targeted therapies in children with radioactive iodine refractory disease. A large long-term study reported for the first time on risk of secondary malignancies in patients with differentiated thyroid carcinoma treated with radioactive iodine. New fundamental data elucidated the T3 mediated mechanisms of hepatocyte differentiation and interactions of thyrocytes, thyroid stromal microenvironment and immune cells during Hashimoto thyroiditis. Several clinical studies add to current knowledge on congenital hypothyroidism and will result in more individualized treatments. Last but not least, new guidelines for treatment of pediatric Graves' disease provide a concise summary of recommendations for optimal care of this patient group.

Mechanism of the year

3.1. Neonatal thyroxine activation modifies epigenetic programming of the liver

Fonseca TL, Garcia T, Fernandes GW, Nair TM, Bianco AC
Nat Commun. 2021 Jul 21;12(1):4446.
doi: 10.1038/s41467-021-24748-8. PMID: 34290257

Brief Summary: Jaundice is a major clinical sign of congenital hypothyroidism. LT4 treatment in hypothyroid neonates normalizes jaundice rapidly by inducing hepatocyte maturation. This mouse model study investigated the molecular mechanisms of thyroid hormone induced hepatocyte differentiation. A postnatal activation of type 2 deiodinase (Dio2) resulted in a T3 surge between postnatal day 1 and 10, inducing permanent hepatocyte specific gene expression mediated by DNA demethylation.

In many developing organs, the local T3 concentration is kept low during fetal development. Differentiation in several organs has been linked to upregulation of tissue specific DIO2 expression and increase in tissue T3 concentration (e.g. retina) [1]. Liver specific Dio2 inactivation (Alb-D2KO) resulted in low T3 signaling during postnatal day 1 and 5, and led to *de novo* DNA hypermethylation. Consequently, the Alb-D2KO liver showed permanently reduced chromatin accessibility in 1551 promoters, and 2426 intragenic regions, and 1363 downregulated genes compared to control liver tissues. Besides reduced chromatin accessibility at promotors, the authors also found evidence for disturbed long-distance chromatin interactions being involved in such fundamental changes of gene expression in Alb-D2KO livers.

In summary, these data provide important insights into the Dio2 mediated postnatal T3 surge, which was found to induce epigenetic regulation and hepatocyte differentiation. They extend current knowledge on the role of DNA demethylation for liver specific gene expression [2].

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2. Postnatal DNA demethylation and its role in tissue maturation. Reizel Y, Sabag O, Skversky Y, Spiro A, Steinberg B, Bernstein D, Wang A, Kieckhaefer J, Li C, Pikarsky E, Levin-Klein R, Goren A, Rajewsky K, Kaestner KH, Cedar H. *Nat Commun.* 2018 May 23;9(1):2040. doi: 10.1038/s41467-018-04456-6. PMID: 29795194.

3.2. Congenital hypothyroidism and hyperthyroidism alters adrenal gene expression, development, and function

Patyra K, Löf C, Jaeschke H, Undeutsch H, Zheng HS, Tyystjärvi S, Puławska K, Doroszko M, Chruściel M, Loo BM, Kurkijärvi R, Zhang FP, Huang CJ, Ohlsson C, Kero A, Poutanen M, Toppari J, Paschke R, Rahman N, Huhtaniemi I, Jääskeläinen J, Kero J
Thyroid. 2022 Apr;32(4):459-471.
doi: 10.1089/thy.2021.0535. PMID: 35044245

Brief Summary: This study combines animal model and patient cohort data on the effect of hyperthyroidism and hypothyroidism at birth on adrenal gland development and function. The data revealed reciprocal effects of neonatal hyper- and hypothyroidism on adrenal development, activity of the adrenal steroidogenic pathway and the adrenal medulla.

In the mouse model, neonatal hyperthyroidism increased adrenal weight by X-zone hypertrophy, while hypothyroidism impaired X-zone development. T4 levels correlated with adrenal weight. Gene expression was altered at two months of age. Genes involved in cholesterol synthesis and catecholamine synthesis were highly up-, or downregulated by neonatal hyperthyroidism, respectively. In contrast, expression levels of genes involved in steroidogenesis were only slightly downregulated in both, hyper- and hypothyroidism. Then, the authors investigated 17-hydroxyprogesterone levels in 14 patients with congenital hypothyroidism and found a mild correlation of TSH and 17-hydroxyprogesterone levels. In a cohort of 73 patients with premature adrenarche, they observed correlations between TSH and dehydroepiandrosterone levels.

Thyroid hormones play important roles in development of different organs. Recent animal and patient data suggested relevant effects of thyroid hormones on adrenal development and function and the pituitary-adrenal axis in patients and in the animal model [1, 2]. The authors documented the effect of thyroid hormones on adrenal development in detail. However, human adrenal development does not seem to be as dependent as hepatocyte differentiation (see paper 3.1 in this chapter) on normal thyroid hormone levels.

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3.3. Extended absorption of liothyronine from poly-zinc-liothyronine: results from a phase 1, double-blind, randomized, and controlled study in humans

Dumitrescu AM, Hanlon EC, Arosemena M, Duchon O, Ettleson M, Giurcanu M, Bianco AC
Thyroid. 2022 Feb;32(2):196-205.
doi: 10.1089/thy.2021.0304. Epub 2021 Dec 31. PMID: 34641706

Brief Summary: This phase 1, double-blind, randomized, single-dose, placebo-controlled, crossover study compared pharmacokinetics, pharmacodynamics, incidence of adverse events, and sleep pattern between the routinely used L-triiodothyronine (LT3) and a newly developed metal-coordinated form of LT3 (poly-zinc-liothyronine, PZL) in 12 healthy volunteers (4 women, 8 men, aged 18-50 years with normal thyroid function). PZL showed more stable pharmacokinetics compared to LT3.

Combination therapy of levothyroxine (LT4) together with LT3, or LT3 alone, may improve symptoms of hypothyroidism and quality of life in adult patients [1,2,3]. However, LT3 administration results in a supraphysiological peak in the first 6 hours after intake associated sometimes with adverse effects, limiting its routine use. A recent consensus statement of the international Thyroid Associations (ATA, ETA, BTA) proposed further research on LT4 + LT3 combination drugs [1].

PZL is a newly developed LT3 prodrug with sustained-release due to a supramolecular metal-coordinated complex. The poly-zinc metal complex of the prodrug extends the intestinal transit time and allows slow release of LT3. This study provides convincing pharmacokinetic-pharmacodynamic data on PZL. The concentration peak after intake of PZL is 30% lower, but maintains T3 levels at a higher level after 12 hours of intake. The T3 serum profile of PZL was not associated with differences in heart rate, blood pressure, or sleep, nor further adverse events compared to LT3 or placebo.

In summary, this small phase 1 study provides very promising results on the pharmacokinetic-pharmacodynamic properties of PZL. Such a new drug has the potential to improve wellbeing of hypothyroid patients. As LT3 treatment has also been used in congenital hypothyroidism, PZL might be of interest for pediatric endocrinologists as well [4]. Larger randomized controlled studies in patients with hypothyroidism are eagerly awaited.

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Thyroid Development

3.4. Transcriptomic signature of human embryonic thyroid reveals transition from differentiation to functional maturation

Dom G, Dmitriev P, Lambot MA, Van Vliet G, Glinoer D, Libert F, Lefort A, Dumont JE, Maenhaut C

Front Cell Dev Biol. 2021 Jun 11;9:669354.

doi: 10.3389/fcell.2021.669354. eCollection 2021. PMID: 34249923

Brief Summary: This *in vitro* study analyzed the transcriptome of the developing thyroid gland in human embryonic thyroids compared to non-thyroidal human embryonic tissues and adult thyroid and adult non-thyroidal tissues. They identified four differently regulated sets of genes co-regulated during thyroid development belonging to different gene ontology groups.

This study covers the key developmental window of thyroid structural and functional differentiation characterized by folliculogenesis and onset of thyroid hormone synthesis [1,2]. The methodological approach to compare embryonic thyroid tissues not only to adult thyroid tissues but to pools of non-thyroidal tissues of the embryo and the adult as control provide a unique insight into dynamics of gene expression changes during thyroid development. The authors identified four major groups of gene expression patterns when comparing the four tissue samples: 1) functional genes upregulated during thyroid differentiation and further increased expression until the adult stage (e.g. thyroglobulin, thyroperoxidase), 2) regulatory genes upregulated during embryonic stages and maintained expression in the adult thyroid (e.g. known transcription factors like *FOXE1*, *HHEX*), 3) possible transient thyroid regulators with highest expression level in the embryonic tissue (e.g. *IGF1*, *FGF10*), and genes of thyroid maturation with low expression in embryonic thyroid but high expression in adult thyroid tissues (e.g. *DIO1*, *CLIC6*). These sets of genes belonged to different gene ontology groups, providing evidence that structural and functional differentiation of the thyroid is a complex process combining angiogenesis, cell polarization and adhesion and cellular maturation as a basis for onset of thyroid hormone synthesis.

This exhaustive data set is an important basis for further research on normal and pathologic thyroid development in the context of congenital hypothyroidism.

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Follow-up Paper from the 2021 Yearbook

3.5. Optimal thyroid hormone replacement dose in immune checkpoint inhibitor-associated hypothyroidism is distinct from Hashimoto's thyroiditis

Mosaferi T, Tsai K, Sovich S, Wilhalme H, Kathuria-Prakash N, Praw SS, Drakaki A, Angell TE, Lechner MG

Thyroid. 2022 May;32(5):496-504.

doi: 10.1089/thy.2021.0685. Epub 2022 Mar 31. PMID: 35199588

Brief Summary: This retrospective monocenter case-control study assessed levothyroxine requirements to achieve euthyroidism defined as two consecutive normal TSH measurements (0.3-4.7 mIU/L) separated by $\geq$ 6 weeks in adult patients with immune checkpoint inhibitor (ICI) associated permanent hypothyroidism (cases) compared to patients with Hashimoto thyroiditis or athyreosis (controls). Patients with ICI-associated hypothyroidism required higher LT4 doses than patients with Hashimoto thyroiditis to achieve normal biochemical thyroid parameters.

ICI-associated hypothyroidism is the consequence of drug-induced destructive thyroiditis by ICI, such as anti-programmed cell death 1 (anti-PD-1), anti-programmed cell death ligand 1 (anti-PD-L1), or anti-cytotoxic T lymphocyte-associated protein 4 (anti-CTLA-4) molecules [1]. In the Yearbook 2021, we highlighted possible genetic factors modulating the risk for ICI-associated hypothyroidism [2].

This study focuses on treatment. Cases and controls were identified in the electronic medical records of the center. Patients with ICI-associated hypothyroidism (n = 103) were identified, of whom n = 66 achieved a stable euthyroid state under LT4 substitution therapy over more than 6 consecutive weeks and were classified as cases (71% post anti-PD1 treatment). Controls were n = 118 patients with antibody proven Hashimoto thyroiditis associated hypothyroidism, and n = 74 patients with athyreosis post-surgery, or post-radioactive iodine ablation. Patients with ICI-associated hypothyroidism required higher LT4 doses than patients with Hashimoto thyroiditis, but comparable doses to athyreotic patients.

This study adds important new understanding of ICI-associated hypothyroidism by direct comparison to the two further major groups of adult patients with hypothyroidism. These data suggest a rapid destruction of the thyroid tissue compared to Hashimoto thyroiditis, rendering the patients functionally athyreotic as after thyroidectomy. The results may lead to a disease stratified replacement dose at diagnosis and during follow-up in this patient group.

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Congenital Hypothyroidism

3.6. Cognitive and white matter microstructure development in congenital hypothyroidism and familial thyroid disorders

Perri K, De Mori L, Tortora D, Calevo MG, Allegri AEM, Napoli F, Patti G, Fava D, Crocco M, Schiavone M, Casalini E, Severino M, Rossi A, Di Iorgi N, Gastaldi R, Maghnie M

J Clin Endocrinol Metab. 2021 Sep 27;106(10):e3990-e4006.

doi: 10.1210/clinem/dgab412. PMID: 34105732

Brief Summary: This observational monocenter study analyzed cognitive scores of children with permanent (n = 28, with athyreosis, ectopy or hypoplasia) *vs.* transient (n = 11, with thyroid gland *in situ*) congenital hypothyroidism (CH) compared to healthy children ('controls', n = 39). The authors found differences in cognitive outcomes and brain MRI white matter microstructure of children with permanent CH despite early diagnosis and treatment with LT4 according to recommended guidelines [1].

The primary aim of neonatal screening and early high dose treatment of newborns with CH is to protect their neurocognitive outcomes. This study provides for the first-time detailed data on neurocognitive outcome in combination with radiological analysis of white matter microstructure in a representative cohort of patients with permanent or transient CH, compared to healthy children. CH was diagnosed at a median age of 12.5 (5-32) days and 21.5 (7-35) days for permanent *vs.* transient CH, respectively. The following items of neurocognitive testing were lower in patients with permanent CH: processing speed, index, sustained visual attention, reading speed, written calculation, and numerical knowledge. In all CH patients, neurocognitive outcome was lower in the presence of either family history of thyroid disease or a mother with Hashimoto thyroiditis. Anomalies of white matter microstructure investigated by brain MRI at the age of 9 years correlated with neurocognitive deficits.

This important study provides quantitative radiological data on brain microstructure in combination with detailed neurocognitive data on children with permanent or transient CH confirming data from a smaller study [1]. These results stress the importance of thyroid hormones for normal brain development and indicate that even early treatment of permanent severe CH might not completely prevent neurocognitive deficits later in life. Finally, they identified an important clinical, yet unrecognized risk factor for impaired neurocognitive outcome: familial and maternal thyroid disease. Whether optimal control of thyroid function before and during pregnancy in affected mothers could prevent this effect, remains to be shown in larger studies.

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3.7. Treatment of congenital hypothyroidism: comparison between L-thyroxine oral solution and tablet formulations up to 3 years of age

Vigone MC, Ortolano R, Vincenzi G, Pozzi C, Ratti M, Assirelli V, Vissani S, Cavarzere P, Mussa A, Gastaldi R, Di Mase R, Salerno M, Street ME, Trombatore J, Weber G, Cassio A

Eur J Endocrinol. 2021 Nov 30;186(1):45-52.
doi: 10.1530/EJE-20-1444. PMID: 34714772

Brief Summary: This retrospective multicenter study examined the biochemical and neurocognitive outcomes of n = 254 patients with congenital hypothyroidism (CH) at age 3 years, treated with either LT4 drops (n = 117) or LT4 tablets (n = 137). Overall, neurocognitive outcome was not different between the two treatment groups. Patients treated with LT4 drops showed lower TSH values at days 15 and 30 suggesting higher risk of overtreatment.

This study compared the effectiveness of LT4 drops to LT4 tablets on biochemical and neurocognitive outcome in patients with CH. The two treatment groups represented the whole range of CH severity (mild to severe, according to guidelines) and thyroid phenotype (athyreosis, ectopy, thyroid gland *in situ*). Median age at diagnosis was comparable (12 days for LT4 drops and 13 days for LT4 tablets, respectively). TSH, FT4 and LT4 mcg/kg/d were compared at 15 days, 1,3,6,12, 24, and 36 months. Those treated with LT4 drops were more likely to have TSH $<$ 0.5 mIU/L at 1 and 6 months of age and FT4 $>$ 2.2 ng/dL at 1 month of age, indicating higher risk for overdosing on equivalent LT4 doses. Total development quotient did not differ between groups at 36 months. However, those treated with LT4 drops had lower scores at 12 months for speech, and at 36 months for eye-hand coordination.

This important study is the first large trial comparing two different LT4 preparations for the treatment of CH. Biochemical and neurocognitive outcomes at 3 years of age were comparable with relevant differences concerning risk of overdosing in the first months of life. This aspect is of clinical relevance in the light of recent data suggesting

not only under- but overdosing is associated with impaired neurocognitive outcomes [1,2]. Therefore, the authors recommend a lower starting dose if using LT4 drops rather than with LT4 tablets.

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2. Long-term effects of L-thyroxine therapy for congenital hypothyroidism. Rovet JF, Ehrlich RM. *J Pediatr.* 1995 Mar;126(3):380–6. doi: 10.1016/s0022-3476(95)70452-3. PMID: 7869196.

3.8. Transient *vs* permanent congenital hypothyroidism in Ontario, Canada: predictive factors and scoring system

Marr A, Yokubynas N, Tang K, Saleh D, Wherrett DK, Stein R, Bassilious E, Chakraborty P, Lawrence SE

J Clin Endocrinol Metab. 2022 Feb 17;107(3):638-648.
doi: 10.1210/clinem/dgab798. PMID: 34726229

Brief Summary: This large monocenter retrospective cohort study (n = 469) of patients diagnosed with congenital hypothyroidism (CH) identified predictors of transient *vs.* permanent CH. The authors developed a 4-item risk score (0-13 points) to be used from the age of 12 months on to predict transient CH during follow-up based on 1) screening TSH, 2) current LT4 dose (mcg/kg/d), 3) presence or absence of maternal thyroid disease, and 4) TSH values over the upper reference range between 6-12 months of age.

The incidence of transient CH has increased over the last two decades. Current guidelines recommend reevaluating patients without definitive permanent CH (athyreosis, hypoplasia, ectopy) between ages 2 to 3 years. Patients with thyroid gland *in situ* and a LT4 dose requirement < 3.0 mcg/kg/d at age 6 months may be reevaluated from age 6 months onwards [1,2]. The current work adds to these recommendations a detailed multivariable logistic regression analysis in the so far largest CH cohort reported in this context. The authors performed 3 analyses: 1) all patients (n = 469) without imaging result, 2) all patients with imaging result (n = 404), and 3) only patients with gland *in situ* (n = 159). First, in all patients (thyroid dysgenesis and gland *in situ*), they show the LT4 dose cut-off level < 3.0 mcg/kg/d at age 12 months has 74% sensitivity and 83% specificity for transient CH. Second, they developed a 4-item risk score (without imaging results) to predict transient CH and successful weaning of LT4 beyond the age of 12 months.

These data are important in the context of the increasing incidence of transient CH. The risk score provides a simple but robust prediction to assess the likelihood of transient *vs.* permanent CH beyond the age of 12 months and will help counsel parents whether and at what age weaning off LT4 therapy might be safe for their child.

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2. Early Determinants of Thyroid Function Outcomes in Children with Congenital Hypothyroidism and a Normally Located Thyroid Gland: A Regional Cohort Study. Saba C, Guilmin-Crepon S, Zénaty D, Martinerie L, Paulsen A, Simon D, Storey C, Dos Santos S, Haignere J, Mohamed D, Carel JC, Léger J. *Thyroid.* 2018 Aug;28(8):959–967. doi: 10.1089/thy.2018.0154. Epub 2018 Jul 30. PMID: 29968521.

New Genes

3.9. Upregulation of GBP1 in thyroid primordium is required for developmental thyroid morphogenesis

Yang RM, Zhan M, Zhou QY, Ye XP, Wu FY, Dong M, Sun F, Fang Y, Zhang RJ, Zhang CR, Yang L, Guo MM, Zhang JX, Liang J, Cheng F, Liu W, Han B, Zhou Y, Zhao SX, Song HD

Genet Med. 2021 Oct;23(10):1944-1951.
doi: 10.1038/s41436-021-01237-3. Epub 2021 Jun 30. PMID: 34194003

Brief Summary: This genetic and developmental study identified pathogenic mutations in *GBP1*in patients with congenital hypothyroidism investigated by exome sequencing. In the zebrafish model, knockdown experiments revealed hypothyroidism and disordered thyroid morphology. These data suggest *GBP1* as a new candidate gene for thyroid dysgenesis.

Pathologic structural differentiation of the thyroid gland results in thyroid dysgenesis and congenital hypothyroidism. Using exome sequencing, the authors identified mutations in *GBP1* in 3 of 98 patients with congenital hypothyroidism. Then, the authors performed extensive developmental studies to investigate the functional mechanisms of the identified *GBP1* mutations in the zebrafish model. They revealed disordered thyroid gland morphogenesis characterized by reduced thyroid surface and reduced follicle number. They further showed that wild-type *GBP1* was able to rescue the pathologic thyroid phenotype. Finally, they showed that GBP1 has a role in inhibiting cell adhesion during thyroid morphogenesis thus promoting follicle formation.

This interesting paper proposes a new candidate gene for thyroid dysgenesis and confirms that tightly coordinated cell-adhesion processes are fundamental for normal thyroid morphogenesis [1,2].

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3.10. GWAS of thyroid dysgenesis identifies a risk locus at 2q33.3 linked to regulation of Wnt signaling

Narumi S, Opitz R, Nagasaki K, Muroya K, Asakura Y, Adachi M, Abe K, Sugisawa C, Kühnen P, Ishii T, Nöthen MM, Krude H, Hasegawa T

Hum Mol Genet. 2022 May 10:ddac093.
doi: 10.1093/hmg/ddac093. Online ahead of print. PMID: 35535691

Brief Summary: This genome-wide association study (GWAS) of patients with thyroid dysgenesis identified a genetic risk locus for thyroid athyreosis and ectopy. In depth genetic analyses of the disease associated region suggested a new disease mechanism of thyroid dysgenesis mediated by impaired Wnt pathway signalling.

To elucidate the pathogenesis of thyroid dysgenesis, the authors performed GWAS in well-phenotyped Japanese patients with thyroid dysgenesis (n = 142). They identified a risk locus at 2q33.3 for thyroid athyreosis and ectopy and confirmed its relevance in an independent German patient cohort (n = 80 patients with thyroid dysgenesis) conferring an overall risk (OR) of 2.23 for thyroid dysgenesis, and of 3.17, and 3.12 for athyreosis and ectopy, respectively. Combined *in silico* epigenome, transcriptome, genotype-tissue expression, and chromatin interaction studies revealed that the risk locus contained cis-regulatory sequences of two genes of the Wnt pathway (*FZD5*, and *CCNYL1*). Higher expression of these two genes was associated with the risk genotype, suggesting enhanced Wnt signalling as a possible disease mechanism for thyroid athyreosis and ectopy, consistent with recent data in a zebrafish model [1].

This first GWAS on two homogenous cohorts of patients with thyroid dysgenesis suggests a new genetic pathological mechanism for thyroid dysgenesis, extending current knowledge on the molecular basis of congenital hypothyroidism.

Reference

1. Enhanced Canonical Wnt Signaling During Early Zebrafish Development Perturbs the Interaction of Cardiac Mesoderm and Pharyngeal Endoderm and Causes Thyroid Specification Defects. Vandernoot I, Haerlingen B, Gillotay P, Trubiroha A, Janssens V, Opitz R, Costagliola S. *Thyroid.* 2021 Mar;31(3):420–438. doi: 10.1089/thy.2019.0828. Epub 2020 Sep 16. PMID: 32777984.

Autoimmune Thyroid Disease

3.11. Lymphocyte infiltration and thyrocyte destruction are driven by stromal and immune cell components in Hashimoto's thyroiditis

Zhang QY, Ye XP, Zhou Z, Zhu CF, Li R, Fang Y, Zhang RJ, Li L, Liu W, Wang Z, Song SY, Lu SY, Zhao SX, Lin JN, Song HD
Nat Commun. 2022 Feb 9;13(1):775.
doi: 10.1038/s41467-022-28120-2. PMID: 35140214

Brief Summary: This *in vitro* study identified interactions between distinct cell populations of the thyroid gland and immune cells in the context of Hashimoto thyroiditis. The authors provide important new insights into the pathological mechanism of Hashimoto thyroiditis by characterizing the stromal microenvironment promoting lymphocyte infiltration, and immune cell subpopulations involved in thyrocyte destruction.

The authors used single cell-RNA sequencing of thyroid tissue and peripheral mononuclear cells of patients with Hashimoto thyroiditis to analyze the cells participating in the stromal microenvironment of the thyroid gland. They identified 3 distinct cell populations (ACKR1+ endothelial cells, CCL21+ fibroblasts, and CCL21+ myofibroblasts) in the stromal microenvironment of the thyroid gland during Hashimoto thyroiditis. Further, they analyzed cell-cell communication based on ligand-receptor interactions between stromal cells and immune cells and revealed that the 3 stromal subpopulations than thyrocytes were responsible for promoting immune cell infiltration. Comparing immune cell populations of peripheral blood and thyroid, the authors observed inflammatory dendritic cells and macrophages being only present in the thyroid gland. These cells expressed high levels of IL-1beta, possibly promoting thyrocyte apoptosis.

In summary, these fundamental immunological studies provide an in-depth overview and extend our current knowledge on cellular interactions of thyrocytes, thyroid stromal cells and immune cells during Hashimoto thyroiditis [1,2].

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3.12. The 2022 European Thyroid Association guideline for the management of pediatric Graves' disease

Mooij CF, Cheetham TD, Verburg FA, Eckstein A, Pearce SH, Léger J, van Trotsenburg ASP
Eur Thyroid J. 2022 Jan 1;11(1):e210073.
doi: 10.1530/ETJ-21-0073. PMID: 34981748

The 2022 European Thyroid Association Guidelines for the management of Graves' disease in the pediatric age group is an important document summarizing all aspects of the disease, ranging from diagnosis, medical treatment with its advantages and side effects, definitive treatment by thyroidectomy or radioiodine ablation in the child, endocrine orbitopathy, and thyroid cancer risk. These guidelines provide a concise summary of the current knowledge in the field of Graves' disease in the child with evidence-based recommendations highly relevant for daily clinical practice.

Paediatric Thyroid Cancer

3.13. NTRK and RET fusion-directed therapy in pediatric thyroid cancer yields a tumor response and radioiodine uptake

Lee YA, Lee H, Im SW, Song YS, Oh DY, Kang HJ, Won JK, Jung KC, Kwon D, Chung EJ, Hah JH, Paeng JC, Kim JH, Choi J, Kim OH, Oh JM, Ahn BC, Wirth LJ, Shin CH, Kim JI, Park YJ

J Clin Invest. 2021 Sep 15;131(18):e144847.
doi: 10.1172/JCI144847. PMID: 34237031

Brief Summary: This retrospective analysis of clinical, pathologic, and genetic characteristics of n = 106 children with differentiated thyroid carcinoma (DCT) showed that fusion oncogene associated papillary thyroid carcinoma (PTC) is more frequent in young children and is associated with larger tumors, extrathyroidal extension and metastases. They showed decreased radioiodine uptake, which could be successfully restored by selective fusion-targeted therapy.

This important work reveals insights into the genetic alterations in pediatric PTCs: First, fusion oncogenes are very frequent (92.9%) in children aged < 10 years, compared to children aged 10-15 years (27.5%) and 15-20 years old patients (13.5%). In contrast, PTC due to *BRAF* mutations increased in frequency with age (7.1%, 30.0%, and 65.4%, respectively in children < 10 years, 10-15 years, and 15-20 years). Second, comparing PTC due to fusion oncogenes *vs. BRAF* mutations, fusion oncogene PTCs showed larger tumors, and larger extrathyroidal extension with lymph nodes, and lung metastases with low radioiodine uptake. Gene expression profiles of fusion oncogene PTCs revealed higher degree of dedifferentiation associated with higher expression of MAPK pathway genes, but lower expression of the sodium/iodide symporter gene (*SLC5A5*), which encodes the molecular basis for radioiodine uptake in the thyrocyte. Based on these results, the authors treated two young children (ages 4 and 7 years) with radioiodine-refractory lung metastases with fusion-targeted therapy and achieved in both patients restored radioiodine uptake of the lung metastases.

These data are of high clinical relevance for treatment of pediatric patients with PTC providing evidence that 1) molecular testing needs to be integrated in routine diagnostics of pediatric PTCs, and 2) PTCs caused by fusion oncogenes can be treated successfully with fusion-targeted therapies. These results were confirmed in a second large study (see paper 3.14 in this chapter) [1].

Reference

1. Fusion Oncogenes Are Associated With Increased Metastatic Capacity and Persistent Disease in Pediatric Thyroid Cancers. Franco AT, Ricarte-Filho JC, Isaza A, Jones Z, Jain N, Mostoufi-Moab S, Surrey L, Laetsch TW, Li MM, DeHart JC, Reichenberger E, Taylor D, Kazahaya K, Adzick NS, Bauer AJ. *J Clin Oncol.* 2022 Apr 1;40(10):1081–1090. doi: 10.1200/JCO.21.01861. Epub 2022 Jan 11. PMID: 35015563.

3.14. Fusion oncogenes are associated with increased metastatic capacity and persistent disease in pediatric thyroid cancers

Franco AT, Ricarte-Filho JC, Isaza A, Jones Z, Jain N, Mostoufi-Moab S, Surrey L, Laetsch TW, Li MM, DeHart JC, Reichenberger E, Taylor D, Kazahaya K, Adzick NS, Bauer AJ

J Clin Oncol. 2022 Apr 1;40(10):1081-1090.
doi: 10.1200/JCO.21.01861. Epub 2022 Jan 11. PMID: 35015563

Brief Summary: This retrospective monocenter study of clinical, pathological and molecular analysis of n = 113 children with differentiated thyroid cancer (DTC) showed that genetic analysis of DTC provided more accurate information on prognosis and outcome than classical histological categorization alone. Larger tumors and higher metastasis rate were observed in patients with fusion oncogene DCT than in patients with *BRAF* mutations.

In analogy to the previous paper (3.13) in this chapter [1], the authors analyzed tumor biology in the context of molecular analysis in children with DTC. They showed differences between patients with *BRAF* mutations *vs.* fusion oncogenes for 1) lymph node metastases (17% *vs*. 60%), distant metastases (0% *vs.* 17%), and persistent disease at 1 year of follow-up (17% *vs.* 36%). Further, they showed that 91% of patients age < 10 years carried fusion events, while the prevalence of *BRAF* mutations increased in patients between 10-20 years.

In summary, these results are in accordance with the data in the previous paper in this chapter stressing the importance of systematic molecular analysis in pediatric patients with DTC for better prediction of tumor biology and prognosis, and possible fusion oncogene targeted therapies. For this, prospective larger studies are planned, and their results eagerly awaited.

Reference

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3.15. Association between radioactive iodine treatment for pediatric and young adulthood differentiated thyroid cancer and risk of second primary malignancies

Pasqual E, Schonfeld S, Morton LM, Villoing D, Lee C, Berrington de Gonzalez A, Kitahara CM

J Clin Oncol. 2022 May 1;40(13):1439-1449.
doi: 10.1200/JCO.21.01841. Epub 2022 Jan 19. PMID: 35044839

Brief Summary: This retrospective study showed that children and young adults (< 45 years) treated with radioactive iodine (RAI) for primary Differentiated Thyroid Cancer (DTC) have increased risks for leukemia and solid cancers, particularly beyond 20 years after treatment.

This retrospective study provides data on the risks of secondary cancer and leukemia after RAI treatment for DTC in childhood or young adulthood in 36,311 patients diagnosed between 1975-2017. Increased relative risk of solid malignancy after RAI was 1.23. Higher relative risk was observed in patients who were younger at DTC treatment (RR 1.6 in patients with RAI before age 25 years). The cumulative incidence of second solid malignancy at 20 years after DTC was 5.6% with RAI *vs.* 5.0% without RAI and increased continuously with age. In this large cohort of DTC patients post-RAI, the authors estimated that, 14% of hematological malignancies, 6% of solid tumors and 5% of breast cancer were associated with prior RAI.

These important data provide for the first time a reliable estimate of the relative risk for second malignancies in DTC survivors treated with RAI during a long follow-up period. They further confirm previous data on risk of leukemia [1]. This work as well as a second paper from last year [2] opens the discussion on risk and benefits of RAI, especially in low-risk DTC in young patients.

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2. Radioactive Iodine Treatment for Children and Young Adults with Thyroid Cancer in South Korea: A Population-based Study. Seo GH, Kong KA, Kim BS, Kang SY, Moon BS, Yoon HJ, Kim HO. *J Clin Endocrinol Metab.* 2021 Jun 16;106(7):e2580–e2588. doi: 10.1210/clinem/dgab192. PMID: 33755732.

4. Growth and Growth Factors

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Preface

The majority of papers included in this selection of articles report clinical studies with potential impact on the diagnostic work-up and treatment of children with growth disorders. Two Cochrane systematic reviews, analyzing data on efficacy and safety of rhGH therapy in children with X-linked hypophosphatemia and cystic fibrosis, show that the evidence supporting such therapy is still insufficient. A prospective observational study in children with chronic kidney disease undergoing kidney transplantation (KT) suggests a positive effect of rhGH treatment prior to KT on long-term growth outcome after KT. A study in adults with Prader-Willi syndrome confirms what is already recognized in children, rhGH therapy seems to have no adverse impact on respiration and sleep parameters. A multi-center, prospective, randomized, double-blind, early intervention study evaluating the effects on growth of two different oral nutritional supplements administered for 90 days to children aged 24-48 months, suggests that early nutritional intervention represents a feasible option for improving growth in children of low-income countries. A randomized controlled trial in boys with constitutional delay of growth and puberty shows that testosterone increases growth velocity independently of aromatisation to estrogens. With regard to novel treatments for children with short stature, the era of long-acting GH (LAGH) in clinical practice is approaching. Two studies report positive results in terms of both efficacy and safety of two different weekly LAGH formulations. Next generation DNA sequencing application to children with severe short stature is providing novel insights into the genetics of human growth. Herein, two studies show the high prevalence of even unexpected genetic variants in children with short stature and minor skeletal anomalies as well as in children with suspected GH insensitivity. Finally, the discovery of a different impact of IGF1R signaling on cardiac function according to age, positive in early life and progressively detrimental with age, and the report of *CRK* haploinsufficiency as the first example of a genetic disorder affecting both GH and IGF signaling, represent new paradigms in normal and aberrant growth.

Important for Clinical Practice

4.1. Recombinant growth hormone therapy for X-linked hypophosphatemia in children

Smith S, Remmington T

Cochrane Database Syst Rev, 2021. 10: p. CD004447
PMID: 34618915

Brief Summary: This systematic review examined the effects of rhGH treatment in children with X-linked hypophosphatemia. Despite a significant improvement in height SDS from baseline, evidence from the 2 identified studies was limited to support its clinical use in these patients.

X-linked hypophosphatemia (XLH) is an inherited disorder of phosphate homeostasis, caused by mutations in the *PHEX* gene, which encodes a membrane-bound endopeptidase expressed in bones and teeth. XLH is the most common cause of inherited phosphate wasting and is characterized by rickets and osteomalacia, disproportionate short stature, hypophosphatemia, abnormal phosphate reabsorption and altered vitamin D metabolism.

Conventional treatment, based on oral phosphate and calcitriol supplementation does not always normalize serum phosphate concentrations and linear growth. Therapy with recombinant human growth hormone (rhGH) therapy has been suggested for improving linear growth but results are conflicting, some showing acceleration of growth velocity and improvement of phosphate retention and bone mineral density, but some others showing a worsening of the pre-existent disproportionate stature [1].

This systematic review assessed the efficacy and safety of rhGH treatment in children with XLH. Linear growth, mineral metabolism, endocrine function, renal function, bone mineral density, body proportions and the incidence of adverse effects were the outcome measures taken into consideration. It included randomized controlled studies (both published and unpublished) in children aged 0-18 years treated with rhGH alone or in combination with calcitriol and oral phosphate, compared with either placebo or conventional treatment alone.

Based on the selection criteria only 2 studies (comprising only 20 patients) (2,3) could be included. One was a cross-over study with 5 participants (Seikaly 1997, 2) and the second was a 3-year study with 15 participants (Živicnjak 2011, 3). Seikaly et al. randomized patients to receive either placebo or rhGH (0.08 mg/kg/daily) for 12 months, and then crossed-over to the other study arm for a further 12 months. Živicnjak et al. gave rhGH 0.4 mg/kg once a week for 3 years whilst the control group received no additional treatment and no placebo (Živicnjak 2011). Both studies reported a significant improvement in height SDS from baseline with rhGH. However, the review authors found no difference in height SDS after three years of treatment. Seikaly et al. reported significant increase in phosphate levels after 3 months of rhGH but it was not maintained at 6, 9 and 12 months. Improvement in TmP/GFR was reported in the cross-over Seikaly study after 3 months of rhGH treatment, but the changes after 6, 9, and 12 months were not significantly different from baseline. Živicnjak et al. found a transient increase in TmP/GFR in the rhGH treated group but these results were not confirmed upon re-analysis. Therefore, the efficacy of rhGH on renal function in terms of TmP/GFR remains uncertain as well as change in alkaline phosphatase levels.

Neither study reported a change in urinary calcium to creatinine ratio between the rhGH treated or control groups. No difference in sitting height, arm and leg length SDS was found after one year in the rhGH treated as compared to the control group in Živicnjak study. The treatment was overall well-tolerated.

In conclusion, data on the efficacy and safety of rhGH therapy in children with XLH were insufficient to provide recommendations for clinical practice. Although rhGH therapy may be potentially beneficial to children with XLH, its clinical use cannot be recommended on the basis of the available evidence.

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4.2. Recombinant growth hormone therapy for cystic fibrosis in children and young adults

Thaker V, Carter B, Putman M

Cochrane Database Syst Rev. 2021 Aug 23;8(8):CD008901.

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PMID: 34424546

Brief Summary: This systematic review evaluated the efficacy and safety of rhGH therapy in improving lung function, quality of life and clinical status of children and young adults (aged up to 23 years) with cystic fibrosis (CF). Eight randomized and quasi-randomized controlled trials, collectively comprising 291 patients, were included. Short-term rhGH treatment (duration ranging from 6 to 12 months) improved anthropometric parameters (height, weight and lean body mass), with no dose-dependent effect. There was no robust evidence that rhGH treatment improved lung function, muscle strength, or quality of life. rhGH therapy increased fasting blood glucose levels, although without crossing the threshold for diagnosis of diabetes.

Cystic fibrosis (CF) is a multi-system monogenic autosomal recessive disease, caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene which encodes the CFTR protein that regulates the transport of electrolytes such as chloride and bicarbonate (1). This chronic disease severely affects lungs, digestive system and pancreas. A further hallmark of CF is poor ponderal and linear growth, closely related to lung function, nutritional status and overall health status. Multiple factors are implicated in growth impairment of CF patients. Inflammation, malnutrition and glucocorticoid treatment affect GH/IGF1 axis function (2). rhGH therapy has been proposed as a therapeutic option but the evidence supporting this use of rhGH is weak. According to a previous systematic review, short-term rhGH treatment improved height, weight, growth rate, bone mineral content and pulmonary function without adverse effects such as diabetes (3).

The current review suggests that rhGH therapy may be effective in improving anthropometric parameters such as height, weight and body mass index in children with CF whereas no consistent benefit was observed in pulmonary function, quality of life and clinical status. Though no increased incidence of diabetes was found, the observed significant rise in glucose levels raises concern about long-term detrimental effect on glucose homeostasis in patients predisposed to CF-related diabetes.

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4.3. Growth hormone treatment in the pre-transplant period is associated with superior outcome after pediatric kidney transplantation

Jagodzinski C, Mueller S, Kluck R, Froede K, Pavicic L, Gellermann J, Mueller D, Querfeld U, Haffner D, Zivicnjak M

Pediatr Nephrol, 2022. 37(4): p. 859-869

PMID: 34542703

Brief Summary: This prospective observational cohort study investigated growth rate after kidney transplant in children with chronic kidney disease (CKD) and growth failure, who received or did not receive rhGH treatment before transplantation. Patients pre-treated with rhGH showed better growth rates with taller height SDS at 7 years after transplantation. Positive effects of pre-transplant rhGH therapy were also observed on transplant function, inflammation, anemia. These data reinforce the indication to rhGH treatment in the pre-transplant period in CKD patients with short stature, with effects that span beyond improvement of growth.

Advanced stages of CKD are associated with disproportionate short stature with preferential impairment of leg growth as a consequence of the CKD related mineral and bone disorder (CKD-MBD). Kidney transplantation (KT) is the therapy of choice in CKD stage 5 but catch-up growth after KT is usually limited, with 40% of patients experiencing reduced adult height despite successful KT. Many factors influence post-KT growth outcomes, such as age, parental height, birth size, degree of growth retardation, transplant function and steroid exposure [1,2].

The achievement of an optimal height at the time of KT significantly influences adult height and can be reached by a careful control of caloric intake, metabolic and electrolyte homeostasis. Treatment with rhGH is proven to improve growth in short children with CKD stages 3–5 [3]. Discontinuation of rhGH therapy at the time of KT is standard practice, as it may raise the risk of transplant rejection and thereby impair long-term graft function.

Monitoring of spontaneous growth after KT for at least 12 months before considering rhGH treatment is the currently recommended strategy to optimize post-transplant linear growth [4]. Whether treatment with rhGH prior to KT has long-term effects on growth after KT had not been investigated.

The objective of this prospective observational cohort study was to evaluate post-transplant growth in 146 prepubertal kidney allograft recipients who received rhGH treatment prior to KT (n = 52) or not (n = 94). GH therapy was initiated at a median age of 1.93 years, continued over a median period of 1.23 years and was stopped in all patients at the time of KT. Mean height z-scores at the time of KT did not differ between children with or without prior rhGH treatment (median duration of the treatment 1.23 years). Post-KT rhGH treatment was initiated in 18% of patients without prior rhGH treatment but in none of the prior rhGH treated group. Nevertheless, post-transplant growth was significantly higher in the latter group, with the maximum difference in stature between the 2 groups observed 7 years after KT (mean height − 0.85 SDS in patients treated with rhGH before KT versus − 1.76 SDS in those who did not receive rhGH before KT, $p < 0.05$). The improvement in height SDS in the rhGH treated group was mainly related to a more pronounced increase in leg length in early post-transplant years. Notably, non-prior rhGH treatment was associated with a faster decline in transplant function, lower hemoglobin, higher C-reactive protein and higher steroid exposure.

In conclusion, rhGH treatment in prepubertal children with CKD before KT resulted in superior long-term growth outcomes after KT compared to patients not exposed to rhGH treatment. Furthermore, positive effects on inflammation, anemia and preservation of transplant function were observed in the pre-KT rhGH treated patients. These data encourage treatment with rhGH in the pre-transplant period in CKD patients presenting with persistent short stature.

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4.4. Effects of growth hormone treatment on sleep-related parameters in adults with Prader-Willi syndrome

Shukur HH, Hussain-Alkhateeb L, Farholt S, Nørregaard O, Jørgensen AP, Hoybye C

The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 9, e3634–e3643

PMID: 33950234

Brief Summary: This trial explored the effects of rhGH treatment in patients with Prader-Willi syndrome (PWS) on respiratory and sleep parameters. The trial was randomized and placebo-controlled for 1 year, followed by a 2-year, open-phase GH treatment period. Polysomnography performed every 6 months revealed no adverse effects of rhGH treatment on respiratory parameters such as apnea-hypopnea index and tonsillar hypertrophy. Sleep efficiency improved after rhGH treatment. These findings support the use of rhGH treatment in adults with PWS.

PWS is considered the most frequent genetic cause of obesity, occurring in approximately 1:10,000-1:30,000 live births and is a complex multisystem disorder, characterized by neonatal hypotonia and feeding difficulties in early infancy, short stature, behavioral problems, cognitive impairment, psychiatric illness, dysmorphic features, multiple endocrine abnormalities, early development of hyperphagia with food-seeking behavior and progressive development of severe obesity, unless eating is not promptly restricted (1). Sleep-related breathing disorders (SRBDs) are frequent in PWS with a high prevalence of obstructive sleep apnea (OSA), up to 80% in children and 22% in adults (2). There are speculations about potential associations between GH therapy and unexpected death in children and for this reason a close monitoring of sleep apneas during GH treatment is needed. Recent data have shown that GH can be safely administered in children with PWS, provided that SRBDs are monitored and treated appropriately (3). In adult PWS patients data were still conflicting.

This study describes the effect of short- and long-term GH treatment on respiratory and sleep parameters in 37 adult PWS patients (15 males and 22 females; mean age 29.5 years). They were randomly assigned to 1 year of GH treatment (n = 19) or placebo (n = 18), followed by 2 years of GH treatment to all. Polysomnography was performed every six months. At year 1, IGF-1 increased in the GH-treated group, whereas it was unchanged in the placebo group. No differences were seen between GH and placebo on respiratory or sleep parameters. Sleep efficiency increased during 3 years of continuous GH treatment, even after adjustment for BMI. Apnea-hypopnea index was normal at baseline and did not increase during treatment. Ear-nose-throat examination did not find any tonsillar enlargement. The authors concluded that GH treatment of adults with PWS was associated with improved sleep efficiency and no adverse impact on respiratory function.

This study confirms in adults what is already recognized in children with PWS, GH therapy has no negative impact on respiration and sleep parameters. However, due to the high frequency of SRBDs, repeated sleep analysis should be performed in any child and adult with PWS, regardless of GH treatment. A weakness is the small number of patients enrolled in the trial but this limitation is partly compensated by the prospective longitudinal long-term placebo-controlled design.

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4.5. Oral nutritional supplementation improves growth in children at malnutrition risk and with picky eating behaviors

Khanna D, Yalawar M, Saibaba PV, Bhatnagar S, Ghosh A, Jog P, Khadilkar AV, Kishore B, Paruchuri AK, Pote PD, Mandyam RD, Shinde S, Shah A, Huynh DTT

Nutrients. 2021 Oct 14;13(10):3590.
PMID: 34684591

Brief Summary: This multi-center, prospective, randomized, double-blind study reports the effects of two different oral nutritional supplements (ONS) on growth in 'picky eaters' children at risk of malnutrition in India. Anthropometric measures (weight, height, weight-for-height, body mass index (BMI), and mid-upper-arm circumference were evaluated at day 1, 30, 60 and 90. Both types of ONS improved anthropometric measures, with the only exception of height, which showed only a non-significant trend towards improvement (possibly due to the short treatment duration). Early nutritional intervention should be considered for improving growth in children at risk of malnutrition in low-income countries.

This multi-center, prospective, randomized, double-blind study aimed at evaluating the effects on growth of two different oral nutritional supplements (ONS) administered for 90 days to a cohort of young children (age between 24 and 48 months), at risk of malnutrition (defined as a weight-for-height percentile between 3rd and 15th) and defined as picky eaters. ONS1 and ONS2 (PediaSure and PediaSure Advance, respectively, Abbott Healthcare Private Limited, Mumbai, India) had similar nutrient compositions, but ONS1 was milk-based, while ONS2 was lactose-free. Both formulations contained 3 macronutrients with matching levels of protein (at 12% of energy), 28 vitamins and minerals, and the pre-biotic fiber fructo-oligosaccharide. The fat energy percent was higher and carbohydrate energy percent was lower in ONS 2 as compared to ONS 1. The administration of both type of ONS increased weight-for-height percentile, weight and BMI compared to the dietary counseling intervention. A trend toward an improvement in height was also observed in ONS treated children, though not statistically significant.

Nutrition exerts its effects on growth throughout the whole life with major impact occurring in the first year of life. Nutrition in early life has not only an immediate impact on growth but also affects future health (1). The majority of data supporting the link between nutrition and growth derive from low-income countries (2), where approximately 25% of children aged < 5 years, show impaired linear growth secondary to malnutrition.

This study suggests that an early nutritional intervention may be a therapeutic and feasible option for improving growth in low-income countries, such as India. The short intervention period may explain the non-significant effect of ONS on linear growth and so longer-term studies are warranted to test the efficacy on height.

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4.6. Serum testosterone and oestradiol predict the growth response during puberty promoting treatment

Huttunen H, Varimo T, Huopio H, Voutilainen R, Tenhola S, Miettinen PJ, Raivio T, Hero M

Clinical Endocrinology. 2022; 96:220–226.
PMID: 34596269

Brief Summary: This randomized controlled trial evaluated the effects of testosterone and aromatase inhibitor treatment on anthropometric measures, and their correlation with estradiol and testosterone serum levels in boys with constitutional delay of growth and puberty (CDGP), to elucidate the respective roles of the two sex steroid hormones on growth during puberty. The results suggest that testosterone plays a major role in pubertal growth, being the best predictor of growth velocity, although adjuvant action of estrogens appears necessary to optimize growth. Extended trials should be warranted to draw clinical indications on the use of testosterone and aromatase inhibitors in CGDP.

During puberty, the gonadotropic axis plays a pivotal role in linear growth, interacting synergistically with somatotropic axis. Indeed, sex steroids, both androgens and estrogens, induce pubertal growth spurt. Sex steroids stimulate the growth hormone (GH)/insulin growth factor-1 (IGF-1) axis but have also a direct effect on bone and cartilage growth plate (1). The respective importance of testosterone and estrogens in the regulation of pubertal growth acceleration is unclear. Constitutional delay of growth and puberty (CDGP) is a variant of the normal process of growth and represents the most frequent cause of delayed puberty in males. It is typically characterized by normal growth during childhood, prepubertal deceleration of height velocity (HV), delayed bone age (BA) and absence of secondary sexual characteristics. In boys, treatment with testosterone increases serum testosterone, estradiol and IGF-1 concentrations, whereas aromatase inhibitors increase serum testosterone only.

In this randomized controlled trial, 28 boys with CDGP (mean age 14.7 $\pm$ 0.58 years, mean bone age 12.4 $\pm$ 1.1 years, mean testicular volume 3.1 $\pm$ 0.92 ml, mean serum testosterone 2.1 $\pm$ 1.2 nmol/L) were treated for 6 months with either low-dose intramuscular testosterone (T; n = 14; 1 mg/kg/month) or the aromatase inhibitor letrozole (Lz; n = 14; 2.5 mg/day). Anthropometry and serum concentrations of testosterone, estradiol and IGF-1 were assessed at 0, 3 and 6 months. Serum testosterone and estradiol concentrations correlated with growth velocity after 6 months of treatment in both groups, with testosterone being the best predictor of growth velocity for both treatment arms. Each nmol increase in serum testosterone increased growth velocity 2.7 times more in the T group compared to Lz group. Only the boys with serum estradiol $>$ 10 pmol/L had a growth velocity $>$ 8 cm/year.

The authors conclude that testosterone increases growth velocity independently of aromatization to estrogens. However, some level of estrogen is needed to optimize the growth rate during puberty. Serum testosterone one week after the injection and serum testosterone and estradiol three months after the onset of aromatase inhibitor treatment can be used as biomarkers for treatment response. Though these results may have potential clinical impact on the management of children with CDGP, the limited number of participants and the estrogen levels lower than assay sensitivity limits, mitigate the value of the study.

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4.7. Effective gh replacement with once-weekly somapacitan vs daily GH in children with GHD: 3-year results from REAL 3

Sävendahl L, Battelino T, Rasmussen MH, Brod M, Saenger P, Horikawa R

J Clin Endocrinol Metab, 2022. 107(5): p. 1357-1367.
PMID: 34964458

Brief Summary: This multicenter, randomized, controlled, phase 2 study compared the effects of once-weekly long-acting growth hormone (GH) somapacitan versus daily GH administered to GH-deficient (GHD) children over a period of 156 weeks. Efficacy estimates were height velocity (HV; cm/y) at year 3 and changes from baseline to end for height SDS, HV SDS, IGF-I SDS, and insulin-like binding protein-3 (IGFBP-3) SDS. Incidence of adverse events (AEs), bone age advancement, detection of anti-somapacitan and anti-GH antibodies after 3 years, and IGF-I/IGFBP-3 levels were assessed as safety endpoints. Somapacitan showed comparable safety and non-inferiority to daily GH over 3 years, with an improved sustainability for patients and families. Somapacitan appears promising as a valid alternative to daily GH treatment in children with GHD.

Currently approved GH treatment regimens are based on daily injections, which can be burdensome for patients and caregivers, leading to reduced treatment adherence and suboptimal clinical outcomes [1, 2]. Since 1999, long-acting GH (LAGH) formulations have been developed as a potential more bearable alternative for GH treatment, as they can be administered weekly, biweekly, or monthly [3]. Somapacitan, a once-weekly reversible albumin-binding GH derivative, has been approved for the treatment of adults in Europe, United States and Japan, and it is under scrutiny for treatment of children with GHD [4]. This study reports the results from a multicenter, randomized, controlled, phase 2 trial, comparing somapacitan and once-daily GH for 156 weeks (NCT02616562) in prepubertal children with GHD.

Fifty-nine children with GHD were randomized (1:1:1:1 ratio) to receive either once-weekly somapacitan (n = 16,0.04; n = 15, 0.08; n = 14, 0.16 mg/ kg/wk) or daily GH (n = 14, 0.034 mg/kg/d, equivalent to 0.238 mg/kg/wk) subcutaneously during the 26-week main trial period and the 26-week extension trial period. For the 104-week safety extension (total 3 years), all participants who received somapacitan during the first year continued with somapacitan dose of 0.16 mg/kg/wk. Daily GH doses remained unchanged for the whole duration of the trial.

At years 2 and 3, somapacitan at doses of 0.08/0.16 and 0.16/0.16 mg/kg/wk was non-inferior in terms of height velocity (HV) and change of height SDS, as compared to daily GH. During the first year of treatment, IGF-1 and IGFBP-3 levels increased in a dose-dependent manner in the somapacitan treatment arms. After 3 years of treatment, the mean increase in IGF-1 and IGFBP-3 SDS from baseline was similar across the somapacitan-treated and daily GH treated children. IGF-1 levels $> +2$ SDS were recorded in 39.5% of somapacitan treated children vs 28.6% of daily treated children. However, in the high dose somapacitan arm (0.16 mg/kg/wk) 57.1% of children showed IGF-1 $> +2$ SDS at least once during the trial. Of note, mean bone age advancement during the 3 years of the trial was 4.0 and 4.9 SD in the medium and high dose somapacitan arm, respectively (0.08 mg/kg/wk and 0.16 mg/kg/wk) vs 3.0 SD for daily GH. However, bone age/chronological age ratio remained $<$ 1 in all treatment arms during the three years of the trial.

The impact of somapacitan on phsycological measures (emotional and social well-being, and physical functioning) was evaluated using the Growth Hormone Deficiency–Child Impact Measure (GHD-CIM). Total scores favored somapacitan over daily GH, without reaching statistical significance. Somapacitan was well tolerated with no new clinically significant safety or local tolerability issues.

In summary, the results of this phase 2 trial support the non-inferiority and comparable safety of somapacitan to daily GH over 3 years and suggest an improved sustainability in terms of treatment burden for patients and families. Somapacitan appears promising as a valid alternative to daily GH treatment in children with GHD.

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4.8. Weekly lonapegsomatropin in treatment-naive children with growth hormone deficiency: the phase 3 height trial

Thornton PS, Maniatis AK, Aghajanova E, Chertok E, Vlachopapadopoulou E, Lin Z, Song W, Dam Christoffersen E, Breinholt VM, Kovalenko T, Giorgadze E, Korpal-Szczyrska M, Hofman PL, Karpf DB, Shu AD, Michael Beckert M.
J Clin Endocrinol Metab, 2021. 106(11): p. 3184-3195.
PMID: 34272849

Brief Summary: This randomized, open-label, controlled, phase 3 trial compared the effects of once-weekly long-acting GH lonapegsomatropin versus daily GH in GHD children over a period of 52 weeks. Efficacy was evaluated by height velocity (HV) and height gain from baseline to end. The long-acting formulation showed not only non-inferiority but also superior efficacy compared to daily GH. Adverse events (AEs), bone age advancement and immunogenicity were not different between the 2 groups of treatment. Lonapegsomatropin may represent a promising alternative to daily GH treatment in children with GHD.

Lonapegsomatropin is a once-weekly long-acting GH formulation for children and adults with GHD consisting of the parent drug, somatropin, an inert methyoxy polyethelene glycol carrier, and a TransCon® linker (1). This study reports the results of the Phase 3 randomized, open-label, controlled trial comparing weekly lonapegsomatropin to daily somatropin (heiGHt; NCT02781727). The enrolled children with GHD were randomized to receive either once-weekly lonapegsomatropin ($n = 105$; 0.24 mg/kg/wk) or daily rhGH ($n = 56$; 0.034 mg/kg/d) for 52 weeks. Treatment was completed by 104 patients in the lonapegsomatropin arm and by 55 patients in the daily rhGH arm.

At week 52, mean height velocity was higher in children treated with lonapegsomatropin compared with those treated with daily GH (11.2 vs 10.3 cm/year, $p < 0.001$). Mean height gain was 1.10 SDS on lonapegsomatropin vs 0.96 SDS on daily GH ($p = 0.01$). On lonapegsomatropin, IGF-1 levels increased between 0 to $+2$ SDS earlier than on daily GH, and showed a higher average IGF-1 SDS throughout the study. 7.6% of lonapegsomatropin treated patients vs 3.6% of daily GH treated children showed IGF-1 levels $> +2$ SDS at least once during the trial. No notable difference in bone age maturation was observed between the two treatment groups. No increase in adverse events, immunogenicity and local reactions was observed in patients treated with lonapegsomatropin compared to daily somatropin. In the rapidly evolving landscape of long-acting GH formulations, lonapegsomatropin appears as a valid alternative to daily GH treatment in children with GHD.

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New Perspectives

4.9. High prevalence of variants in skeletal dysplasia associated genes in individuals with short stature and minor skeletal anomalies

Sentchordi-Montané L, Benito-Sanz S, Aza-Carmona M, Díaz-González F, Modamio-Høybjør S, De la Torre C, Nevado J, Ruiz-Ocaña P, Bezanilla-López C, Prieto P, Bahíllo-Curieses P, Carcavilla A, Mulero-Collantes I, Barreda-Bonis AC, Cruz-Rojo J, Ramírez-Fernández J, Bermúdez de la Vega JA, Travess AM, González de Buitrago Amigo J, Del Pozo A, Vallespín E, Solís M, Goetz C, Campos-Barros A, Santos-Simarro F, González-Casado I, Ros-Pérez P, Parrón-Pajares M, Heath KE
Eur J Endocrinol, 2021. 185(5): p. 691-705.
PMID: 34516402

Brief Summary: This study evaluated the prevalence of genetic variants in children with idiopathic short stature (ISS) using next-generation sequencing (NGS). Heterozygous variants in known genes involved in skeletal physiology were identified in almost 20% of children. The use of advanced genetic analysis techniques will lead to an increased identification of new genetic variants, expanding the knowledge on the pathogenesis of short stature.

Short stature is the most common cause of referral to pediatric endocrinologist. The application of next-generation sequencing (NGS) to children with unexplained short stature, known as idiopathic short stature (ISS), has unveiled many genetic variants associated with ISS (1). This study assessed the prevalence of genetic variants in a cohort of 108 children with severe ISS (mean height SDS: -2.97) and mild skeletal anomalies. Body disproportion was indicated by arm spam/height ratio (A/H) ratio ≤ 0.96 and/or sitting height/height (SH/H) ratio ≥ 0.55. SHOX mutations were excluded in all patients. A NGS skeletal panel, including *ACAN*, *IHH*, *NPR2*, *FGFR3*, *COL2A1*, and *PTPN11*, was applied. A total of 20 heterozygous variants were identified in 21/108 patients (19.4%), mostly *ACAN* ($n = 10$) and IHH ($n = 7$). Single variants were also found in *COL2A1*, *CREBBP*, *EXT1*, and *PTPN11*. Interestingly, only 2/10 patients with *ACAN* variants had advanced bone age. Out of 20 variants, 11 (55%) were classified as pathogenic and 17 (80.9%) were inherited. No pathogenic or likely pathogenic or VUS were identified in *NPR2* or *FGFR3* genes. Lower limb shortening (determined by the SH/H ratio) and paternal skeletal traits showed a high concordance with NGS results.

This study confirms previous reports showing that a high percentage of children with ISS, and especially those with mild skeletal traits, may harbor variants in genes involved in growth plate physiology. The Authors targeted specific skeletal dysplasia genes, and it is likely that the use of whole exome sequencing (WES) would identify even more genetic variants. The extensive use of WES will certainly lead to identify new genetic variants underlying ISS.

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4.10. Genetic characterization of short stature patients with overlapping features of growth hormone insensitivity syndromes

Andrews A, Maharaj A, Cottrell E, Chatterjee S, Shah P, Denvir L, Dumic K, Bossowski A, Mushtaq T, Vukovic R, Didi M, Shaw N, Metherell LA, Savage MO, Storr HL

J Clin Endocrinol Metab. 2021;106(11):e4716-e4733.
PMID: 34136918

Brief Summary: In this study, 149 children referred for suspected GH insensitivity (GHI) and short stature underwent genetic characterization through different techniques, including whole exome sequencing, targeted gene sequencing and array comparative genomic hybridization (array-CGH). Genetic alterations were identified in 80/149 subjects (54%), of which 45 were affected by GH–IGF-I axis defects and 35 were diagnosed with known genetic syndromes associated with multi-organ involvement. These results highlight the importance of genetic analysis in children with undefined short stature for a targeted management of comorbidities and specific therapeutic approaches.

This study aimed at defining genetic features of 149 short subjects (58% males) with suspicion of growth hormone insensitivity (GHI). The use of an extensive genetic approach (whole exome sequencing, targeted short stature gene panel, candidate gene sequencing and array-CGH) allowed to identify a genetic diagnosis in 54% (80/149) of subjects. Most of the defects (56%) involved the growth hormone-insulin like growth factor-I (GH-IGF-I) axis (*GHR*, *IGFALS*, *IGF1R*) but other genetic anomalies (associated with 3M syndrome, Noonan syndrome, Silver-Russell syndrome or conditions not previously related to GHI) were also identified. Most of the subjects (86%) with a specific genetic diagnosis had IGF-1 deficiency and were shorter than subjects without a recognized genetic abnormality (height: -4.9 vs -3.4 SDS).

The classical and most severe form of GHI syndrome, first described in 1966 and known as Laron syndrome, is caused by monogenic defects in the GH receptor (*GHR*) gene resulting in severe post-natal growth failure as a consequence of IGF-I deficiency. Over the years, genetic defects involving different components of GH-IGF-I axis, such as STAT5B, IGF-I, IGF-II, IGFALS, PAPPA2 have been recognized, expanding the phenotypic spectrum of GHI states (1).

Although *GHR* variants are the most frequent causes of GHI, this study confirms that the genetic spectrum of GHI is much broader than previously recognized. It shows that genetic characterization of children with severe growth retardation of unknown origin may be crucial for identifying rare conditions which require careful follow-up and multi-specialist care and to orientate treatment choice (e.g. avoiding rhGH treatment in conditions with increased tumor predisposition such as Bloom syndrome).

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New Paradigms

4.11. Fine-tuning cardiac insulin-like growth factor 1 receptor signaling to promote health and longevity

Abdellatif M, Trummer-Herbst V, Heberle AM, Humnig A, Pendl T, Durand S, Cerrato G, Hofer SJ, Islam M, Voglhuber J, Ramos Pittol JM, Kepp O, Hoefler G, Schmidt A, Rainer PP, Scherr D, Von Lewinski D, Bisping E, McMullen JR, Diwan A, Eisenberg T, Madeo F, Thedieck K, Kroemer G, Sedej S

Circulation, 2022: Jun 21;145(25):1853-1866
PMID: 35616058

Brief Summary: This translational study evaluated cardiac health and lifespan in two cardiomyocyte-specific transgenic mice with either enhanced or reduced IGF-1 signaling and in human cardiac biopsies from failing and nonfailing hearts. Increased IGF1R expression was related to better cardiac performance in young mice but faster decline of cardiac function with aging. Conversely reduced IGF1R signaling was associated improved lifespan and superior cardiac profile during aging. Human failing hearts showed exaggerated IGF1R signaling. These data unveil a novel role of IGF1R signaling in cardiac health, which acts in a biphasic way according to age and suggest that the use of pharmacological inhibitors of IGF1 could be beneficial in elderly adults at risk of heart failure.

The GH/IGF-1 axis is considered a key regulator of cellular metabolism and aging (1). Previous studies revealed that loss-of-function mutations in the IGF1 receptor (*IGF1R*) or its downstream effectors promote longevity in various organisms. There is evidence that IGF-1 signaling plays a role in the modulation of lifespan. However, the consequence of diminished IGF1 signaling on health span (i.e., the disease-free period of life), with the exception of cancer, remains largely controversial (2). IGF-1 signaling influences cardiac homeostasis. Reduced cardiomyocyte IGF-1 signaling exerts detrimental effects, whereas its activation is linked to enhanced cardiac contractility and physiological hypertrophy (2, 3). In contrast to this assumption, recent studies have reported a positive effect of reduced IGF-1 signaling on age-related cardiac remodeling, and an improvement of cardiac function obtained with IGF1R monoclonal antibodies in aged female mice. Therefore, modulation of IGF1R signaling might be either beneficial or detrimental on cardiac function depending on the organism age.

To test this hypothesis, the authors generated two cardiomyocyte-specific transgenic mice with either enhanced or reduced IGF-1 signaling. The mice were examined at different life stages by multiple structural and functional analyses. Furthermore, the expression of IGF1R in aged human hearts was investigated using left ventricular (LV) biopsies obtained from explanted failing hearts. Increased IGF1R signaling was associated with a superior cardiac function in young mice but this positive effect declined with aging leading to earlier heart failure and reduced lifespan compared to wild-type animals. On the contrary, mice with reduced IGF1R signaling showed inferior cardiac function in early life but a better cardiac function with aging and prolonged lifespan. Consistently, in humans (mean age 60 ± 2 years), failing hearts showed increased IGF1R expression and signaling. These findings suggest that IGF1R signaling has a double-faced impact on cardiac function according

to age, positive in early life and progressively detrimental with age. Aging based pharmacological interventions aimed at modulating IGF1R signaling may be worth testing to improve cardiac function.

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4.12. Crk haploinsufficiency is associated with intrauterine growth retardation and severe postnatal growth failure

Deodati A, Inzaghi E, Germani D, Fausti F, Cianfarani S

Horm Res Paediatr. 2021;94(11-12):456-466.

PMID: 35086092

Brief Summary: This study reports 2 girls with a complex phenotype associated with severe short stature and IUGR who were diagnosed with a de novo 17p13. 3 deletion by array-CGH. The deletion involved the *CRK* gene that transcribes for Crk protein, a component of GH and IGF-I receptor signaling pathways. In vitro assay confirmed defective *CRK* expression and GH/IGF1 signaling in the patients' peripheral blood mononuclear cells. The 2 children were treated with rhGH with a partial response in patient 1 and catch-up growth in patient 2, encouraging the use of rhGH to improve adult height in this condition.

This study reports two girls with a *de novo* 17p13.3 deletion and sharing a common phenotype characterized by intrauterine growth retardation, post-natal severe short stature, facial dysmorphisms and brain MRI abnormalities. The microdeletion harbors the *CRK* gene, which encodes for Crk, an intracellular adaptor downstream both GH and IGF-1 receptors (1, 2). *In vitro* assay analysis performed on PBMCs showed that reduced expression of CRK was associated with impaired GH/IGF-1 signaling, likely accounting for the pre- and post-natal growth failure of the two patients. rhGH treatment was effective in reducing growth deficit in one patient and inducing sustained catch-up growth in the other one.

Multiple pathogenic CNVs have been described in short children with history of intrauterine growth retardation (3). In particular, 17p13.3 anomalies are detected with a relative high frequency in short SGA children, suggesting that genes located in this region could play a key role in pre- and post-natal growth. This region is characterized by genomic instability and has been associated with isolated lissencephaly sequence (ILS) and Miller Dieker syndrome (MDS), characterized by facial dysmorphisms, microcephaly, short stature, seizures, cardiac malformations and different severity grades of cerebral agyria (4).

These findings show that *CRK* haploinsufficiency may represent the first example of a genetic disorder affecting both GH and IGF signaling, ultimately leading to pre- and post-natal growth failure.

Furthermore, the efficacy of rhGH therapy either in reducing growth deficit or stimulating catch-up growth, indicates that rhGH may be a therapeutic option in short children with 17p13.3 deletion and *CRK* haploinsufficiency.

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5. Bone, Growth Plate and Mineral Metabolism

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Introduction

The skeletal research field has produced several important findings during the past year, including advances in the treatment of rare skeletal disorders and an ever deeper understanding of skeletal biology and disorders of mineral metabolism. This year we highlight several advances in the field of skeletal mineralization, ranging from basic mechanisms of tissue mineralization to clinical advances in the understanding of rare disorders characterized by aberrant mineralization.

Translational highlights include several exciting studies including a study demonstrating that retinoic acid inhibition can block hedgehog induced epiphyseal fusion, an article in Endocrinololgy showing that CNP-induced PKA activation promote growth plate chondrogenesis, and study reveling the mechanism by which Fibrillin-1- deficiency promote overgrowth in a mouse model of Marfan syndrome. Novel Advances in skeletal biology feature a Nature article visualizing the conformational changes that turn on and off the the calcium-sensing receptor, a Science article that report that mineralization creates contractile forces in collagen fibrils crucial to the mechanical properties of mineralized bone, and a genetic study explaining the lack of of rare variants to complex trait heritability from SNP-array based genome wide association studies as well as a GWAS show that adult height is predominantly associated with variants in genes that are enriched in the resting zone of the growth plate.

In addition to these areas of progress, the chapter reports several exciting clinical advances including a very large cohort study of fibrous dysplasia reporting fracture rates and fracture risk factors in fibrous dysplasia, a large cohort of patients with ENPP1- or ABCC6-related ectopic calcification and hypophosphatemic rickets, revealing that heterogenous calcification and multiple organ complications occur with both *ENPP1* and *ABCC6* variants, and thus suggesting overlapping pathology.

Novel Treatments for Rare Skeletal Disorders

5.1. Targeting TGF-β for treatment of osteogenesis imperfecta

Song IW, Nagamani SC, Nguyen D, Grafe I, Sutton VR, Gannon FH, Munivez E, Jiang MM, Tran A, Wallace M, Esposito P, Musaad S, Strudthoff E, McGuire S, Thornton M, Shenava V, Rosenfeld S, Huang S, Shypailo R, Orwoll E, Lee B
J Clin Invest. 2022 Apr 1;132(7):e152571.
doi: 10.1172/JCI152571.
Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/35113812/

In brief: Currently, there is no disease-specific therapy for osteogenesis imperfecta (OI) where most children, of all forms of OI, with significant fracture history, are managed by bisphosphonates. Preclinical studies demonstrate that excessive TGF-β signaling is a pathogenic mechanism in OI. In this, phase I study of fresolimumab (TGF-β neutralizing antibody) in 8 adults with OI there were dose-dependent effects on bone mass and turnover.

Commentary: The management of OI typically involves a multidisciplinary approach; currently, there are no approved medical therapies for OI. Treatment of bone fragility is limited to the repurposing of medications that

are used to treat osteoporosis. Bisphosphonates (BPNs) have become the standard of care, especially in children. In adults, the benefits and the consequences of long-term treatment with BPNs are less certain. BPNs however, don't address specific pathogenetic mechanism(s) in OI, and, hence, they have no effect on extraskeletal manifestations. In murine models, excessive TGF-β signaling has been found to be a key driver of pathogenesis and neutralizing TGF-β improves bone mass, bone biomechanical properties, as well as pulmonary abnormalities (1).

A phase I study of fresolimumab, a TGF-β neutralizing antibody, was conducted in 8 adults with OI. Safety and effects on bone remodeling markers and lumbar spine areal bone mineral density (LS aBMD) were assessed.

OI bone demonstrated woven structure, increased osteocytes, high turnover, and reduced maturation. SMAD phosphorylation was the most significantly upregulated molecular event. Gene set enrichment analysis identified the TGF-β pathway as the top-activated signaling pathway, and pathway analyses showed that TGF-β1 was the most significant activated upstream regulator mediating the global changes identified in OI bone. Treatment with fresolimumab was well-tolerated and associated with increases in LS aBMD in participants with OI type IV, whereas participants with OI type III and VIII had unchanged or decreased LS aBMD. No significant adverse events related to the medications were noted.

While promising, treatment with fresolimumab or Losartan as an angiotensin II-receptor agent with anti- TGF-β properties requires investigation in children where bone turnover is higher and previous repurposed medications have not been found to be safe (e.g. denosumab). However, if found effective, anti-TGF-β medications could be the first disease-specific therapy with the potential to significantly impact skeletal and extraskeletal manifestations of OI in children.

Reference

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5.2. Reiterative infusions of MSCs improve pediatric osteogenesis imperfecta eliciting a pro-osteogenic paracrine response: TERCELOI clinical trial

Infante A, Gener B, Vázquez M, Olivares N, Arrieta A, Grau G, Llano I, Madero L, Bueno AM, Sagastizabal B, Gerovska D, Araúzo-Bravo MJ, Astigarraga I, Rodríguez CI

Clin Transl Med. 2021 Jan;11(1):e265.

Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/33463067/

In brief: In order to explore safety, efficacy, and feasibility of repeated infusions of mesenchymal stem cells (MSCs) as a treatment for OI, two pediatric OI patients were treated with repeated infusions of HLA-matched MSCs. The patients tolerated the treatment well and some positive effects on physical functioning and well-being are reported.

Commentary: Infusion of mesenchymal stem cells as a treatment for osteogenesis imperfecta has been considered for more than 20 years. However, enthusiasm has been hampered by findings that engraftment of infused cells into bone is low. Consequently, most studies have focussed on improving the engraftment potential of the infused stem cells.

In this study, two non-immunosuppressed pediatric OI patients were treated with 5 consecutive infusions of related donor MSCs. Both patients tolerated the infusions well with no or minimal side-effects with moderately increased BMD as well as reportedly positive effects on physical functioning and well-being. Due to its small size (two patients) this is obviously a pilot study. Neverthless, both patients tolerated repeated infusion of MHC-matched MSCs well with no immunological side-effects detected and some positive effects on both objective (BMD) and subjective parameters suggest that larger trials would be motivated. This may lead to a new approach using repeated infusions of MSCs isolated from relatives and expanded in vitro as a treatment for moderate and severe OI.

5.3. Safe and persistent growth-promoting effects of vosoritide in children with achondroplasia: 2-year results from an open-label, phase 3 extension study

Savarirayan R, Tofts L, Irving M, Wilcox WR, Bacino CA, Hoover-Fong J, Font RU, Harmatz P, Rutsch F, Bober MB, Polgreen LE, Ginebreda I, Mohnike K, Charrow J, Hoernschemeyer D, Ozono K, Alanay Y, Arundel P, Kotani Y, Yasui N, White KK, Saal HM, Leiva-Gea A, Luna-González F, Mochizuki H, Basel D, Porco DM, Jayaram K, Fisheleva E, Huntsman-Labed A, Day JRS
Genet Med. 2021 Dec;23(12):2443-2447.
Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34341520/

In brief: In achondroplasia, longitudinal bone growth is inhibited resulting in severe, disproportionate short stature. In this open-label extension study of participants from the phase 3 study, daily subcutaneous injection of vosoritide during 104 weeks resulted in increase in growth velocity and a small but significant improvement in body proportions.

Commentary: Achondroplasia is caused by autosomal activating mutation in the fibroblast growth factor receptor 3 gene (FGFR3) resulting in constitutively activation of the mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase pathway in chondrocytes and therefore inhibited endochondral ossification. C-type natriuretic peptide, encoded by NPPC, and its receptor, natriuretic peptide receptor 2 (NPR2), are potent stimulators of endochondral ossification at the level of the growth plate. Vosoritide is a recombinant C-type natriuretic peptide analogue with extended half-life that improve growth in children with achondroplasia and was recently approved for the treatment of achondroplasia in growing children aged 2 years and older.

In this extention study following the placebo-controlled phase 3 study, all 119 children (n = 58 from the active arm and n = 61 from the placebo arm) received subcutaneous vosoritide at a dose of 15.0 μg/kg/day. The study present data at the one year follow-up of the extension study, which corresponds to two years on treatment for children originally randomized to vosoritide and one year on treatment for children who crossed over to vosoritide from placebo. The improvement of growth velocity observed in children treated with vosoritide in the phase 3 study was maintained with an annualized growth velocity of 5.75 ± 1.84 cm/year at week 78 and 5.52 ± 1.77 cm/year at week 104. Children who crossed over from placebo to vosoritide similarly increased their growth velocity to 5.97 ± 1.83 cm/year at week 78 and 5.43 ± 2.03 cm/year at week 104. In addition, a small but significant improvement in body proportions (upper-to-lower body segment ratio) is reported.

The improvement in body proportion is minimal and seems hardly clinically significant. However, from the presented data, it appears that it may be continous and could therefore potentially lead to clinically meaningful improvements of body disproportion with extended duration of treatment. With time, this and other studies will reveal if vosoritide provide other beneficial effects for individuals with achondroplasia.

Advances in Clinical Practice

5.4. PTH infusion for seizures in autosomal dominant hypocalcemia type 1

Sastre A, Valentino K, Hannan FM, Lines KE, Gluck AK, Stevenson M, Ryalls M, Gorrigan RJ, Pullen D, Buck J, Sankaranarayanan S, Allgrove J, Thakker RV, Gevers EF
N Engl J Med. 2021 Jul 8;385(2):189-191.
Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34233101/

In brief: This study retrospectively analysed a cohort of patients with autosomal dominant hypocalcemia type 1 and recurrent hypocalcemic seizures treated with continuous subcutaneous PTH (1-34) infusions using insulin pumps. Compared to conventional therapy, PTH (1-34) infusion resulted in higher mean serum calcium and magnesium, lower mean serum phosphorus and fewer seizures.

Commentary: Autosomal dominant hypocalcemia type 1 (ADH1) is caused by gain-of-function mutations in the calcium-sensing receptor gene (*CASR*). CaSRs are expressed in the parathyroid glands regulate parathyroid hormone (PTH) secretion whereas CaSRs expressed in the kidney regulate calcium reabsorption. Conventional treatment with calcium and vitamin D analogs tend to exacerbate hypercalciuria and increase the risk for nephrocalcinosis and renal complications in patients with ADH1.

The study reports a retrospective review of six ADH1 patients with recurrent hypocalcemic seizures on conventional treatment who were selected for treatment with continuous subcutaneous PTH (1-34) infusions. PTH infusion therapy has previously been assessed in a clinical trial and shown to modestly improve calcium homeostasis and bone turnover markers compared to twice daily PTH (1). In the Sastre A *et al.* study, they found that PTH (1-34) infusion resulted in higher mean serum calcium and magnesium, lower mean serum phosphorus and fewer seizures compared to calcium and vitamin D analogue treatment. However, the variability in serum calcium levels remained high with infusion therapy. One benefit of the current study is that it shows that continuous PTH pump treatment can be feasible in clinical settings and therefore could be considered in selected patients. Even more useful for this group of patients, long-acting PTH and maybe especially calciolytic drugs may soon be available and help improve the management and outcome for patients with ADH1 (2).

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5.5. Long bone fractures in fibrous dysplasia/McCune-Albright syndrome: prevalence, natural history, and risk factors

Geels RES, Meier ME, Saikali A, Tsonaka R, Appelman-Dijkstra NM, Boyce AM

J Bone Miner Res. 2022 Feb;37(2):236-243.

Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34668234/

In Brief: This study characterized a large cohort of patients with fibrous dysplasia/McCune Albright syndrome in order to assess the fracture prevalence and identify risk factors for future factures.

Commentary: Fibrous dysplasia/McCune-Albright syndrome (FD/MAS) is due to mosaic, activating mutations in GNAS, causing aberrant Gαs activation and cAMP production disrupting normal differentiation of bone cell progenitors, resulting in fibrotic leasions in the affected parts of the skeleton. Skeletal disease is typically recognised during childhood, with the final disease burden established during late adolescence or early adulthood. The disease spectrum ranges from minimal disease with small leasions affecting one or a few bones, to severe disease affecting a large proportion of the skeleton leading to fractures, pain, and disability. Due to the rarity and large variability of the condition, the lifetime risk and predictive risk factors have not been well-established.

In the study, two large prospective cohorts were combined (total n = 419 patients) evaluated as part of a natural history study at the Natonal Institutes of Health (Bethesda, MD, USA) (n = 186) or retrospective chart review of the clinical cohort at Leiden University Medical Center (Leiden, the Netherlands) (n = 233). In the combined cohort 59% had MAS (FD + endocrinopathies) with histories of precocious puberty (30%), hyperthyroidism (17%), GH excess (11%), neonatal Cushing syndrome (4%), and hypophosphatemia (28%).

Out of 419 indivduals, 248 (59%) patients suffered one or more fracture (median 1, range 0–70, IQR 4). The median age at first fracture was 8 years (range 1–76, IQR 10) and fracture rates peaked between 6 and 10 years of age and decreased thereafter. Lifetime fracture risk was associated with the fibrous dysplasia burden score ($p < 0.01$) and MAS hyperthyroidism ($p = 0.01$). Both skeletal burden score >25 and age at first fracture ≤ 7 years were associated with a higher total number of lifetime fractures ($p < 0.01$).

The study presents the largest cohort of FD/MAS so far and spans the full lifespan and therefore provides new information on the fracture burden and risk factors for fractures in the FD/MAS population thereby helping clinicians to identify FD/MAS patients at risk for fractures and may be candidates for early therapeutic interventions.

5.6. Vitamin D level and fractures in children and adolescents: a systematic review and meta-analysis

Zheng C, Li H, Rong S, Liu L, Zhen K, Li K

Bone Miner Metab. 2021 Sep;39(5):851-857

Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34115219/

In brief: Vitamin D plays a pivotal role in calcium homeostasis and bone health; a question is therefore frequently raised regarding the risk of fractures in children with vitamin D deficiency. This is more pertinent in safeguarding cases where it is not uncommon to find fractures along with vitamin D deficiency or insufficiency. In this meta-analysis, no relationship between vitamin D levels and the risk of bone fractures in children and adolescents was found.

Commentary: In this meta-analysis, the authors identified 13 studies with 3983 participants <18 years old ($n=2570$ in the control group and $n=1373$ in the fracture group) that reported bone fractures and 25OHD values. Children with metabolic bone disorders including rickets, chronic diseases and prolonged steroid use were excluded. Using the Newcastle–Ottawa scale, 2 articles were of moderate quality and 11 articles were of high quality.

There were no significant differences in 25OHD levels in the control and fracture groups; there was no publication bias ($P = 0.282$ in Egger's test). Sensitivity analysis, OR $= 1.22$ (95% CI: 0.96, 1.56), I2 $= 0\%$, and $P = 0.64$ demonstrated no significant difference in the proportion of subjects with vitamin D deficiency between the fracture and control groups; there was no publication bias ($P = 0.156$ in Egger's test). These analyses suggest that fractures in children may not be related to vitamin D levels. Given that only case-control and cross-sectional studies were included in the analyses (because of the inclusion criteria), large cohort studies and their metanalyses will be required to confirm the role of vitamin D deficiency and insufficiency in fractures in children and adolescents.

5.7. Ectopic calcification and hypophosphatemic rickets: natural history of ENPP1 and ABCC6 deficiencies

Ferreira CR, Kintzinger K, Hackbarth ME, Botschen U, Nitschke Y, Mughal MZ, Baujat G, Schnabel D, Yuen E, Gahl WA, Gafni RI, Liu Q, Huertas P, Khursigara G, Rutsch F

J Bone Miner Res. 2021 Nov;36(11):2193-2202

Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34355424/

In brief: Generalised arterial calcification of infancy (GACI) is clinically and genetically a heterogeneous disorder caused by mutations in *ENPP1*or *ABCC6* variants. This multicentre study identified early mortality risk in GACI patients despite attempts to treat with bisphosphonates, high prevalence of rickets in *ENPP1*, but not *ABCC6* deficiency, and that heterogenous calcification and multiple organ complications occur with both *ENPP1* and *ABCC6* variants suggesting overlapping pathology.

Commentary: Generalized arterial calcification of infancy (GACI) is a rare autosomal recessive disorder characterized by calcification of arteries and marked neointimal proliferation leading to arterial stenoses and cardiac complications. The disease can manifest prenatally and has a mortality of ~55% in the first 6 months of life despite intensive therapy. However, the disease course after the initial 6 months is not well characterized, with reports limited to case series or small retrospective studies.

In this study, 247 patients with GACI (from birth to 58.3 years of age) across 19 countries were reviewed. Overall mortality was 54.7% (13.4% in utero or stillborn), with a 50.4% probability of death before the age of 6 months (critical period).

Despite lack of evidence bisphosphonates, especially first-generation, are routinely used for the management of GACI during infancy. This study, however, found that bisphosphonate treatment had no survival benefit based on a start-time matched analysis and inconclusive results when initiated within 2 weeks of birth.

Similar prevalence of GACI phenotypes between ENPP1 and ABCC6 deficiencies, including arterial calcification (77.2% and 89.5%, respectively), organ calcification (65.8% and 84.2%, respectively), and cardiovascular complications (58.4% and 78.9%, respectively) were found. However, mortality was higher for ENPP1 versus ABCC6 variants (40.5% versus 10.5%, respectively; $p = 0.0157$).

A higher prevalence of rickets was reported in 70.8% of surviving affected individuals with ENPP1 compared to individuals with ABCC6 (11.8%; $p = 0.0001$) variants. Eleven affected individuals presented with rickets and without a GACI diagnosis termed autosomal recessive hypophosphatemic rickets type 2 (ARHR2), all had

confirmed *ENPP1*variants. Approximately 70% of these patients demonstrated evidence of ectopic calcification or complications similar to those seen in individuals with GACI, which shows that ARHR2 is not a distinct condition from GACI but represents part of the spectrum of ENPP1 deficiency.

With the potential for recombinant human (rh)ENPP1-Fc protein to be trialled in GACI and ARHR2, this study will form a benchmark to compare outcomes of novel treatments with historic controls. This study also raises questions about using bisphosphonates in infancy given that it does not offer any survival benefits.

5.8. Growth in achondroplasia including stature, weight, weight-for-height and head circumference from CLARITY: achondroplasia natural history study-a multi-center retrospective cohort study of achondroplasia in the US

Hoover-Fong JE, Schulze KJ, Alade AY, Bober MB, Gough E, Hashmi SS, Hecht JT, Legare JM, Little ME, Modaff P, Pauli RM, Rodriguez-Buritica DF, Serna ME, Smid C, Liu C, McGready J

Orphanet J Rare Dis. 2021 Dec 23;16(1):522.

Abstract: https://pubmed-ncbi-nlm-nih-gov/34949201/

In Brief: Based on over 37,000 length/height, weight and head circumference measures from 1374 patients with achondroplasia in the USA, updated length/height-for-age, weight-for-age, head circumference-for-age (HC) and weight-for-height curves were generated for children up to 18 years of age (HC up to 5 years of age).

Commentary: Growth charts for achondroplasia have been available. However, those were derived from a mix of longitudinal, cross-sectional, retrospective and/or prospectively collected data from populations ranging in size from 23 to 466 subjects, collected as long ago as 1967 and as recently as 2019.

The Achondroplasia natural history study (CLARITY) collected all available retrospective anthropometry data including length/height, weight and head circumference from achondroplasia patients from 4 US skeletal dysplasia centres (Johns Hopkins University, AI DuPont Hospital for Children, McGovern Medical School University of Texas Health, University of Wisconsin School of Medicine and Public Health). Weight-for-age values beyond 3 SD above the mean were excluded from the weight-for-height and weight-for-age curves to create a stricter tool for weight assessment in this population. Over 37,000 length/height, weight and head circumference measures from 1374 patients with achondroplasia from birth through 75 years of age were compiled. Stature and weight data from birth through 18 years of age and head circumference from birth through 5 years of age were used to construct new length/height-for-age, weight-for-age, head circumference-for-age and weight-for-height curves. The cross-sectional presentation of height-for-age data did not demonstrate a substantial pubertal growth spurt in achondroplasia. Head circumference data indicated no difference from all available data and those derived from data excluding those undergoing base of skull decompression and/or ventricular shunting. This study also explored the possibility of secular trends in weight in children by age group and decade of birth and utilized weight-for-age data to screen for extremes in weight that were excluded from their novel, prescriptive weight-for-age and weight-for-length/height curves.

These curves could be utilized in the clinic and research venues to ascertain deviation from normal cranial growth which should prompt further investigation. These data will also enable to identify children with achondroplasia who carry excess weight for their stature. However, the data are from the USA only and even though all ethnic groups were included, similar country-specific studies will be required to ascertain genetic and environmental factors contributing to growth in achondroplasia patients.

Translational Highlights

5.9. PTH and FGF23 exert interdependent effects on renal phosphate handling: evidence from patients with hypoparathyroidism and hyperphosphatemic familial tumoral calcinosis treated with synthetic human PTH 1-34

Ovejero D, Hartley IR, de Castro Diaz LF, Theng E, Li X, Gafni RI, Collins MT

J Bone Miner Res. 2022 Feb;37(2):179-184.

Abstract: https://pubmed-ncbi-nlm-nih-gov/34464000/

In Brief: Fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) both negatively regulate serum phosphate by increasing renal phosphate excretion. The clinical observation that both PTH and FGF23 are needed for adequate renal phosphate handling is confirmed in this experimental patient study.

Commentary: Serum phosphate is primarily regulated by fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) which both exert their main phosphaturic effect by actions in the proximal renal tubule, decreasing the number and activity of sodium-phosphate co-transporters and thereby decreasing renal phosphate reabsorption.

Deficiency of either PTH or FGF23 cause hyperphosphatemia, indirectly suggesting that both PTH and FGF23 are needed for adequate phosphaturic effects and renal phosphate handling.

In order to test whether the phosphaturic effects of PTH and FGF23 are interdependent, 11 patients with hypoparathyroidism, characterized by high blood phosphate despite FGF23 elevation, and 1 patient with hyperphosphatemic familial tumoral calcinosis (FTC), characterized by hyperphosphatemia due to FGF23 deficiency, were treated with synthetic human PTH (1–34). In patients with hypoparathyroidism, PTH treatment resulted in increased renal cAMP with phosphaturic response resulting in normalization of serum phosphate levels. In the FTC patient, PTH treatment also resulted in increased renal cAMP. Hovewer, this did not result in a phosphaturic response or decrease in serum phosphate levels indicating that both FGF23 and PTH are required for adequate renal phosphate handling.

The study provides the first direct evidence for the interdependency of PTH and FGF23 for adequate phosphaturic response of either of the two important phosphate regulating hormones.

5.10. INZ-701 prevents ectopic tissue calcification and restores bone architecture and growth in ENPP1-deficient mice

Cheng Z, O'Brien K, Howe J, Sullivan C, Schrier D, Lynch A, Jungles S, Sabbagh Y, Thompson D

J Bone Miner Res. 2021 Aug;36(8):1594-1604.

Abstract: https://pubmed-ncbi-nlm-nih-gov/33900645/

In Brief: Enzyme replacement therapy with human ENPP1-Fc protein in *Enpp1*$^{asj/asj}$ mice, a murine model of ENPP1 deficiency, restored circulating levels of PPi, prevented clinical manifestations and decreased mortality.

Commentary: ENPP1 deficiency causes generalized arterial calcification of infancy (GACI) and autosomal-recessive hypophosphatemic rickets type 2 (ARHR2) and is associated with low serum inorganic pyrophosphate (PPi). It manifests as ectopic calcification of multiple tissues, neointimal proliferation, premature mortality, impaired growth, and bone deformities.

INZ-701, a human ENPP1-Fc protein, is in clinical development as an enzyme replacement therapy for the treatment of ENPP1 deficiency. The pharmacokinetic and pharmacodynamic profiles and therapeutic effects of INZ-701 were investigated in *Enpp1*$^{asj/asj}$ mice, a murine model of ENPP1 deficiency. *Enpp1*$^{asj/asj}$ mice have undetectable plasma PPi, lower plasma phosphate, and higher FGF23 levels compared with wild-type (WT) mice. *Enpp1*$^{asj/asj}$ mice on the acceleration diet, containing high phosphate and low magnesium, quickly develop clinical signs, including dehydration, rough hair coat, pinned ears, stiffed legs, and hunched back. *Enpp1*$^{asj/asj}$ mice treated with vehicle had aforementioned clinical signs plus severe ectopic calcification in multiple tissues and bone defects, characteristics of the clinical phenotype observed in GACI and ARHR2 patients.

This study showed a durable PPi response for more than 3 days after a single dose of INZ-701. Treatment of ENPP1-deficient mice every other day with INZ-701 for 8 weeks restored circulating levels of PPi, prevented pathological calcification in all tested organs, restored growth parameters, corrected bone defects, improved clinical signs, and decreased mortality in *Enpp1*$^{asj/asj}$ mice, demonstrating the potential of INZ-701 to treat ENPP1 deficiency. These preclinical findings suggest the potential of INZ-701 to treat ENPP1 deficiency, and support normalization of PPi levels as a surrogate marker to predict clinical benefit in the animal model and potentially in patients with ENPP1 deficiency.

5.11. A reference range for plasma levels of inorganic pyrophosphate in children using the ATP sulfurylase method

Bernhard E, Nitschke Y, Khursigara G, Sabbagh Y, Wang Y, Rutsch F
J Clin Endocrinol Metab. 2022 Jan 1;107(1):109-118.
Abstract: https://pubmed-ncbi-nlm-nih-gov/34498693/

In Brief: This study established a standard range of Inorganic Pyrophosphate (PPi) between 2.36 and 4.44 μM (5th-95th percentiles) in the blood plasma of children and adolescents aged 0 to 18 years, using the ATP sulfurylase assay. There was no sex difference and the range is similar to previously reported adult ranges of 2-5 μM/L.

Commentary: PPi is a potent inhibitor of mineralisation. Circulating PPi prevents mineralisation to occur in non-osseous tissues while a higher concentration of Alkaline phosphatase (ALP) in bones breaks down PPi to facilitate mineralisation. PPi is generated from ATP in the presence of the ENPP1 enzyme. ATP Sulfurylase converts PPi to ATP; the assay utilises ATP sulfurylase to convert PPi to ATP, which is then detected by a luciferase/luciferin luminescence detection kit.

The analytical sensitivity of the ATP sulfurylase assay ranged from 0.15 to 10 μM PPi. Inter- and intra-assay coefficients of variability on identical samples were good ($< 10\%$). The standard range of PPi in the blood plasma of children and adolescents aged 0 to 18 years was calculated as 2.36 to 4.44 μM, with a median of 3.17 μM, with no sex difference. PPi plasma levels did not differ significantly between different pediatric age groups.

Besides providing normative data on PPi in children, this assay is now validated for use in children. This assay provides an important biomarker for the reliable diagnosis of generalised arterial calcification in infancy (low PPi) and hypophosphatasia (high PPi). Further experience however will be required with the measurement of PPi to identify any other disorders or factors which can contribute to the concentration of PPi. There is also potential to use PPi measurements for titrating the dose of asfotase alfa in hypophosphatasia where overtreatment can lead to extra-skeletal calcifications.

5.12. Premature growth plate closure caused by a hedgehog cancer drug is preventable by co-administration of a retinoid antagonist in mice

Koyama E, Mundy C, Saunders C, Chung J, Catheline SE, Rux D, Iwamoto M, Pacifici M
J Bone Miner Res 36, 1387–1402. (2021)
Abstract: https://pubmed-ncbi-nlm-nih-gov/33724538/

In brief: Premature growth plate closure under anti-hedgehog treatment is caused by impaired retinoic acid metabolism in growth plate progenitor cells. In this mouse model, administration of retinoic acid receptor blockers rescued the phenotype and normalized growth plate maturation.

Commentary: With the increasing use of pathway inhibitor therapies in paediatric oncology, unintended effects such as affection of growth plate cartilage have become an imminent clinical challenge. In the present study, Koyama et al investigated the mechanism and pharmacologic interventions regarding premature growth plate closure associated with the hedgehog inhibitor Sondegib. In a murine model system, Sondegib decreased growth plate progenitor cell number and proliferation rate. On an expressional level, retinoid metabolism was altered towards higher local levels of retinoic acid, inducing a profound acceleration of growth plate maturation. *In vivo* treatment with a selective retinoic acid inhibitor could entirely rescue the phenotype by normalizing long bone growth. *In vitro,* micromass cultures of embryonic mesenchymal cell marker gene expression such as *Col2a1*, *Col10a1* and *Sox9* as shown by *in situ* hybridisation revealed normalized patterns after coadministration of the Sondegib and a retinoic acid inhibitor.

Koyama et al systematically approached the phenomenon of growth plate progenitor cell depletion under Sondegib treatment. By analysing the treatment effects, hedgehog signalling was identified as crucial regulator of this important cell population mainly by keeping a local state devoid from retinoic acid action. Since the effects of retinoic acid receptor antagonist co-treatments on the oncologic efficacy of hedgehog inhibition has not been tested, the direct clinical relevance of the findings has to be proven in future studies. Nevertheless, these

data add a fascinating chapter to the complex regulation of growth plate progenitor cells which may aid future therapeutic aspects targeting the growth plate.

5.13. C-type natriuretic peptide-induced PKA activation promotes endochondral bone formation in hypertrophic chondrocytes

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Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University, Kyoto, Japan
Endocrinology 163, bqac005. (2022).
Abstract: https://pubmed-ncbi-nlm-nih-gov/35041746/

In Brief: C-type natriuretic peptide (CNP) is known to stimulate enchondral bone formation, but the distinct cellular pathways and cellular targets are unclear. This study used in vivo and in vitro biosensor systems to identify cGMP-induced activation of PKA as a major effect of CNP with growth promoting effects in hypertrophic growth plate chondrocytes.

Commentary: C-type natriuretic peptide (CNP) represents an important anabolic regulator of endochondral bone growth affecting chondrocyte proliferation, hypertrophy and matrix production. Although the stimulation of enchondral bone formation is already clinically used in the treatment of patients with achondroplasia, the precise mechanism and cellular targets within the growth plate are not entirely elucidated.

These authors used FRET-based cGMP and PKA biosensors to show that CNP both increased intracellular cGMP levels and stimulated PKA activity in transfected ATDC5 chondrocytes. In contrast to cGMP stimulation, CNP-induced PKA activation was enhanced during differentiation. Live imaging of growth plates in radial explants of mice expressing a FRET PKA biosensor revealed CNP-associated activation mainly in the hypertrophic zone. Increased growth plate length under CNP treatment was specifically inhibited in hypertrophic cells by PKA-inhibitor cotreatment, indicating a differentiation specific effect of CNP-induced PKA activation.

In summary, Hirota et al identified PKA-activation in the hypertrophic zone as part of the growth-promoting effect of CNP. These data pave the way for future studies on the CNP-induced cross-talk between cGMP and cAMP signaling and interaction with other regulatory pathways.

5.14. Fibrillin-1 deficiency in the outer perichondrium causes longitudinal bone overgrowth in mice with Marfan syndrome

Sedes L, Wondimu E, Crockett B, Hansen J, Cantalupo A, Asano K, Iyengar R, Rifkin D.B, Smaldone S, Ramirez F
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Hum Mol Genet ddac107 (2022)
Abstract: https://pubmed.ncbi.nlm.nih.gov/35567544/

In Brief: Disproportionate tall stature represents a hallmark feature of Marfan syndrome, although specific mechanisms underlying linear bone overgrowth are unclear. This study used an ex vivo model system to identify dysregulation of TGFβ-binding proteins in the outer perichondrium as causative for the bone overgrowth phenotype in fibrillin-1-deficiency.

Commentary: Marfan syndrome is associated with fibrillin-1 deficiency and represents one of the most common genetic causes for tall stature. While the management of cardiovascular complications has improved substantially over the last decades, skeletal manifestations – in particular long bone overgrowth – remain poorly understood and therefore lack causative treatment options.

Sedes et. al combined data on the role of fibrillin-1 in TGF beta signalling as well as evidence on the role of perichondral tissue in the regulation of the beneath growth plate chondrocytes. By use of a conditional knockout system, fibrilin-1 deficiency in various mesenchymal tissues was shown to affect limb length differentially.

Interestingly, growth promoting effects in mice lacking Fibrillin 1 in perichondrium and tendons was exclusively exerted by effects on the hypertrophic zone. Ingenuity pathway analysis of perchondrial RNAseq data allowed an *in silico*prediction of TGF beta signalling to be downregulated in fibrilin-1 knockout animals. Similar to previous findings in aortic tissue, a redcued expression of LTBP3 and 4 exclusively in the outer perichondrium could be demonstrated by IHC. In metatarsal bone cultures, the authors could finally prove a reversal of bone overgrowth in perichondrium/tendon specific fibrillin-1 deficiency by TGF beta treatment.

This study, for the first time, implicates perichondrial tissue in TGF beta signalling mediated overgrowth at the level of the growth plates. In contrast to previous studies on TGFb-receptor defects featuring shorter bones, the particular association of local fibrillin-1 deficiency most likely caused by deficient binding protein expression and complex formation revealed an opposite phenotype resembling tall stature in patients. While a role of TGF beta bioavailability in the affection of growth plate tissue in Marfan syndrome been suggested before, the current study enables a more specific understanding of the mechanisms causing overgrowth and often extreme tall stature in patients with Marfan syndrome.

Advances in Skeletal Biology and Mineral Metabolism

5.15. Asymmetric activation of the calcium-sensing receptor homodimer

Gao Y, Robertson MJ, Rahman SN, Seven AB, Zhang C, Meyerowitz JG, Panova O, Hannan FM, Thakker RV, Bräuner-Osborne H, Mathiesen JM, Skiniotis G
Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA
Nature. 2021 Jul;595(7867):455-459.
Abstract: https://pubmed-ncbi-nlm-nih-gov/34194040/

In brief: Using cryo-electron microscopy, the calcium-sensing receptor (CaSR) is visualized with different ligands demonstrating, in great detail, how calcimimetic drugs lock the CaSR homodimer in an assymetric configuration, exposing one of the two protomers for G-protein coupling, whereas calciolytic drugs promote the symmetric configuration thereby preventing G-protein coupling.

Commentary: The calcium-sensing receptor (CaSR) is a seven-transmembrane receptor that can sense extracellular calcium levels at the cell surface. CaSR regulates calcium homeostasis primarily through its actions in the parathyroid gland and kidneys, where its activation by elevated circulating Ca^{2+} leads to decreases in parathyroid hormone (PTH) secretion and renal tubular Ca^{2+} resorption. Loss-of-function mutations of CaSR cause hypercalcemia as in neonatal severe hyperparathyrodism or familial hypocalciuric hypercalcaemia type 1 (FHH1). Conversely, gain-of-function CaSR mutations cause hypocalcaemia as in autosomal dominant hypocalcaemia type 1 (ADH1). Clinically, calcimimetic medications e.g. cinacalcet, are used for the treatment of FHH1, while calcilytic drugs currently are in clinical trials for the treatment of ADH1.

The authors expressed near-full-length human CaSR and produced cryo-electron microscopy images that show CaSR bound to Ca^{2+} and different calcilytic or calcimimetic drug molecules. The study visulizes how receptor activation results in asymmetry in the homodimer and therefore G-protein coupling and the specific positions where calcimimetic drugs bind and stabilize the asymmetric, active configuration as well as where and how calciolytic drugs bind and lock the receptor in its symmetric, inactive configuration. The study provides detailed images of the fundamental allosteric mechanisms that control CaSR signalling through G-protein. These findings will aid in the design of improved molecules for targeting of the CaSR and potenially also for other G-protein coupled receptors.

5.16. Mineralization generates megapascal contractile stresses in collagen fibrils

Hang Ping, Wolfgang Wagermaier, Nils Horbelt, Ernesto Scoppola, Chenghao Li, Peter Werner, Zhengyi Fu, Peter Fatzl
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Science 2022 Apr 8;376(6589):188-192.
Abstract: https://pubmed-ncbi-nlm-nih-gov/35389802/

In Brief: The unique composition of organic and inorganic components allows bone to have remarkable biomechanical properties. This *in vitro* study reveals the phenomena of large contractile forces during intrafibrillar mineralization explaining the unusual mechanical properties of bone tissue.

Commentary: Collagen represents the most abundant structural protein in the body and the main organic component of bone. While the structure of bone has been revealed in detail, biomechanical features like collagen fibril prestressing have not been investigated in detail yet.

Ping et al used unmineralized turkey tendons as collagen matrix and, among others, SrCO3 as main mineral for investigations of mineralization. By chemically enabling intrafibrillar collagen mineralization, the group developed an *in vitro* system for monitoring and imaging of contractile stress by in-operando x-ray scattering. Under specific mineralizing conditions, turkey tendon slices developed contractile stress to a maximum of 7.8 MPa, correlating with formation of intrafibrillar crystals. Importantly, this immense contractile force was only achieved if minerals nucleated within the fibrils, not if extrafibrillar deposition occurred. In-operando Raman analysis, small-angle x-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) allowed detailed characterization of the kinetics of intrafibrillar SrCO3 nucleation and the mechanism of prestress formation. Interestingly, the stress of collagen fibrils has shown to be transferred to the nanocrystals themselves, leading to compressional forces parallel to the fibrils as high as 20-40 MPa.

Prestressing is a common strategy to enhance material properties, found both in nature and in material sciences. In line, the authors compared the observed chemomechanical effects to the principle of reinforced concrete using prestressed steel, enabling the deflection of cracks or inhomogeneities in the mineral component. The detailed characterization of prestressing of collagen fibres adds substantially to the understanding of biomechanical properties of mineralizing tissues, in particular bone. Future efforts on tissue and material engineering could highly benefit from the gained insights on chemomechanical effects of collagen based structures.

5.17. Assessing the contribution of rare variants to complex trait heritability from whole-genome sequence data

Wainschtein P, Jain D, Zheng Z

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Nat Genet. 2022 Mar;54(3):263-273.

Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/35256806/

In brief: Genome-wide association studies (GWAS) on unrelated individuals rely on the correlation of common SNP polymorphisms with nearby causal gene variants. In contrast to classical pedigree-based studies, SNP-based approaches only capture a part of the heritability of traits and diseases. This study estimated the heritability of height and BMI based on whole-genome sequence data and identified insufficient SNP tagging of rare variants in low linkage disequilibrium as a main cause for the missing heritability in common SNP approaches.

Commentary: With the increasing number of genomes available for research, GWAS has become a key method to identify genetic variants associated with diseases and phenotypes. Nevertheless, the mapping of all common SNPs as used in current studies is still unable to explain the heritability predicted by pedigree based studies. While this decreased sensitivity is well-know, hypotheses on the reasons for this evidence gap have not been proven so far.

Wainschtain et al used a large number of genomes with European ancestry for SNPs and insertion-deletions, and estimated a SNP-based heritability of 0.5-0.56 for adult height. In order to identify the causes for the gap to the expected total estimate of 0-7-0.8, grouping of minor allele frequencies and linkage distance was performed, increasing heritability estimates for height to 0.70. This discrepancy could mainly be explained by rare variants (low MAF) in low linkage distance. Further, the group investigated the enrichment in heritability for high or low impact on the protein and showed that rare, low-LD and protein-altering variants are more enriched for trait heritability than non-coding variants.

This highly sophisticated approach impressively shows a potential methodological cause for the reported missing heritability of traits and diseases in SNP-based GWAS studies. Importantly, the identified rare variants in low linkage distance could only be covered by use of whole genome sequencing (WGS) due to their lack of coverage in SNP arrays. Especially for polygenic diseases, this study strongly supports the use of WGS in large sample sizes for increased potential to detect causal rare variants.

5.18. Genes with specificity for expression in the round cell layer of the growth plate are enriched in genome wide association study (GWAS) of human height

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J Bone Miner Res 36, 2300–2308. (2021)
Abstract: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/34346115/

In brief: The genetic basis of human body height is only partly understood. This study combined genome-wide association study (GWAS) data with expression data from specific chondrocyte populations to identify genes responsible for skeletal growth. They found strong associations of height variability and genes expressed in early differentiation stages.

Commentary: Research on the genetic regulation of human height is a complex area: lack of human growth plate material, numerous involved regulatory pathways and species-specifc features of the growth plate are among the main burdens of a better understanding of the genetics of body height variability.

Renthal et al. used GWAS data from 700,000 individuals (from the GIANT consortium) and correlated height-associated loci with expression data of specific chondrocyte differentiation stages, derived from both micro dissected murine growth plates and from a mesenchymal cell line.

As a proof of concept, the authors could show an association with height in the GWAS data with a set of 287 genes associated with skeletal disorders. By linking the expression profiles of differential stages of growth plate chondrocytes with GWAS data, the authors could for the first time prove that height is predominantly associated with variants in genes enriched in the reserve zone (stated as the 'round layer'). Further, they confirmed the association of height with genes specific for early differential stages by using RNAseq data from an immortalized mesenchymal cell line. Interestingly, this association was only in part driven by genes linked to monogenic skeletal disorders. Thus, the data emphasizes the importance of general progenitor chondrocytes physiology in the variability of human linear growth. The identified genes revealed enrichment in several expression pathways including Wnt signalling, PTHrP, TGFb and matrix proteins as major superfamilies.

This study impressively illustrates the potential of large GWAS datasets in combination with gene expression data from tissues of interest, in particular in separate differentiation stages as demonstrated with growth plate chondrocytes. Although the different approaches using expression data from growth plates and from a cell line do not show perfect overlaps, high levels of association of early differentiation stage-specific genes with height-associated loci in the GWAS data could be identified. With the increasing availability of GWAS datasets, similar approaches could spur future efforts to identify critical regulatory genes and potential treatment targets.

6. DSD and Gender Incongruence

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Preface

In the past 12 months, the search for "Differences of Sexual Development" or "disorders of sex development" or "ambiguous genitalia" or "gonadal development" or "DSD" in PubMed yielded 625 publications. A similar search for gender incongruence revealed > 325 papers on a search between "transgender and hormones". Among those, 16 are summarized in this chapter. The selection process has been very challenging given the space available but we prioritized the key publications chosen on the quality of methodology, the significance of the outcome, and particularly the impact on clinical practice. We have endeavored to balance basic research and clinical articles. We hope these selected publications will help with understanding and improve both knowledge and the clinical care of patients.

Sex Hormone Replacement Therapies in DSD

6.1. Pubertal induction and transition to adult sex hormone replacement in patients with congenital pituitary or gonadal reproductive hormone deficiency: an Endo-ERN clinical practice guideline

Nordenström A, Ahmed SF, van den Akker E, Blair J, Bonomi M, Brachet C, Broersen LHA, Claahsen-van der Grinten HL, Dessens AB, Gawlik A, Gravholt CH, Juul A, Krausz C, Raivio T, Smyth A, Touraine P, Vitali D, Dekkers OM

Eur J Endocrinol. 2022 Apr 21;186(6):G9-G49.

PMID: 35353710, DOI: 10.1530/EJE-22-0073.

Brief Summary: This Endo-European Reference Network guideline was endorsed by the European Society for Pediatric Endocrinology, the European Society for Endocrinology, and the European Academy of Andrology. It provides extensive data on the hormonal management in young individuals with hypogonadism or DSD in need of treatment to induce or sustain puberty. A systematic literature search was conducted, and the evidence was graded according to the Grading of Recommendations, Assessment, Development, and Evaluation system.

Pubertal induction or sex hormone replacement to sustain puberty is required in adolescents with all forms of hypogonadism including DSD. These patients may be identified in the neonatal period because of atypical genitalia, lack of mini puberty, or may be diagnosed in the puberty/adolescence period when pubertal development is delayed, incomplete, absent, or atypical. The assessment of the hypothalamo-pituitary-gonadal axis includes the quantification of serum concentrations of gonadotropins, FSH, and LH, as well as gonadal sex steroids estradiol and testosterone. In addition, inhibin B, AMH, and insulin-like factor 3 may add useful information about the gonadal Sertoli and Leydig cell function, respectively. AMH is also used as a marker of ovarian reserve. This guideline sheds light on when and how to induce and monitor puberty, and maintenance of puberty by gonadal sex steroids namely testosterone or estradiol. To this end, important data and recommendations are also provided about the use of aromatase inhibitors and gonadotropin treatments. The expert panel recommends that pubertal induction or sex hormone replacement should be individualized but considered at 11 years in girls and 12 years in boys. Psychological aspects of puberty and fertility issues are especially important to address in individuals with sex development disorders or congenital pituitary deficiencies. The transition of these young adults highlights the importance of a multidisciplinary approach, discussing both medical issues and social and psychological issues that arise in the context of these chronic conditions.

The overall aim of treatment is to ensure secondary sexual characteristics and physical and neuropsychological maturation at a similar pace to peers, achieving somatic and psychological well-being, also in the longer perspective bone, cardiovascular, hematopoietic, sexual, and metabolic health.

6.2. Metabolic effects of estradiol versus testosterone in complete androgen insensitivity syndrome

Auer MK, Birnbaum W, Hartmann MF, Holterhus PM, Kulle A, Lux A, Marshall L, Rall K, Richter-Unruh A, Werner R, Wudy SA, Hiort O

Endocrine. 2022 Jun;76(3):722-732.

PMID: 35258786, DOI: 10.1007/s12020-022-03017-8.

Brief Summary: In this multicentre, randomized, double-dummy, double-blind crossover trial the authors investigated differences in metabolic parameters between the individuals with complete androgen insensitivity syndrome (CAIS) receiving testosterone versus estradiol replacement therapy. This is the first study investigating these different treatment options in CAIS. It finds no major differences in metabolic and safety parameters between the treatments.

CAIS is the most common 46, XY DSD, and is characterized by complete loss of androgen receptor function due to X-linked recessive mutations within the androgen receptor gene. The testosterone concentrations are in the normal-to-upper male reference range, while estradiol concentrations are normal to slightly increased relative to male references originating primarily from testicular secretion and peripheral aromatization of androstenedione and testosterone. However, despite aromatization, estradiol concentrations are usually below the usual female reference range.

The researchers analyzed data from 17 females with CAIS who undertook a two-month run-in phase with estradiol, then either received transdermal estradiol followed by crossover to transdermal testosterone or vice versa. After six months, differences in lipids, fasting glucose, insulin, hematocrit, liver parameters, and blood pressure between the treatment phases were investigated. Although it may seem unlikely that testosterone should exert any distinct effect from that of estradiol in females with CAIS, the authors could show that testosterone replacement is non-inferior to estradiol in terms of quality of life and results in comparable levels of estrogens. Both treatments resulted in a less favorable lipid profile, as there was a significant increase in total and LDL-cholesterol and a significant decrease in HDL-cholesterol. In addition, there was a slight but significant increase in BMI in both groups.

The results did not reveal major differences according to treatment for the investigated outcomes. Furthermore, this study provides a different point of view in that testosterone treatment may have beneficial effects on wellbeing and sexual functioning in these individuals. The underlying hypothesis is that testosterone is not only metabolized to estradiol but also to neurosteroids that exert their activity neither via estrogen - nor androgen receptors. Further studies may address the potential differences of both treatments assuming that there is a difference between a systemic increase in estradiol in comparison to a local increase of estradiol via aromatization of testosterone depending on the distribution of aromatase expression in the corresponding target tissues such as bone.

Co-Morbidities Associated with DSD

6.3. Vascular dysfunction and increased cardiovascular risk in hypospadias

Lucas-Herald AK, Montezano AC, Alves-Lopes R, Haddow L, Alimussina M, O'Toole S, Flett M, Lee B, Amjad SB, Steven M, Brooksbank K, McCallum L, Delles C, Padmanabhan S, Ahmed SF, Touyz RM

Eur Heart J. 2022 Mar 17:ehac112.

PMID: 35296881, DOI: 10.1093/eurheartj/ehac112.

Brief Summary: This translational study explored the molecular and cellular mechanisms whereby testosterone impacts vascular function. The findings suggest that hypospadias is associated with vascular dysfunction and represents a risk factor for hypertension and cardiovascular disease in adulthood due to impaired Rho kinase- and Nox5/ROS-dependent signalling.

Low circulating testosterone concentration is an independent determinant of endothelial dysfunction in men. Testosterone influences vascular reactivity by regulating vascular smooth muscle cell $Ca2^+$ channel expression and activity, intracellular $Ca2^+$ homeostasis, Nox-derived reactive oxygen species (ROS) generation, nitric oxide (NO) production, Rho kinase activation, and mitogen-activated protein kinase phosphorylation. Hypospadias develops secondary to reduced antenatal androgen exposure in the majority of 46,XY boys. The authors hypothesized that, similar to adults, lack of androgens during the critical masculinization programming period of foetal development coincides with the time of vasculature development. This androgen deficiency could cause endothelial dysfunction and vascular injury early in life and predispose to hypertension and cardiovascular events in adulthood.

To test the hypothesis, this study investigated clinical and ex-vivo markers related to vascular function. Small subcutaneous arteries and vascular smooth muscle cells from penile skin of adolescent boys undergoing hypospadias repair and controls were isolated for functional studies. Vascular smooth muscle cells were used to assess: Rho kinase, reactive oxygen species (ROS), nitric oxide synthase/nitric oxide, and DNA damage. Compared to controls, adolescents with hypospadias had higher systolic blood pressure, pulse pressure, and carotid intima-media thickness increased vasoconstriction and reduced vasorelaxation. Markers for vascular and systemic oxidative stress and Rho kinase activity were increased in adolescents with hypospadias. Based on the data on admission for cardiometabolic diseases in 6797 men with hypospadias compared with 8073 controls, men born with hypospadias were at increased risk of arrhythmia, hypertension, and heart failure.

Overall, the findings of this study demonstrate an important extragonadal function of testosterone in cardiovascular (patho)physiology, and that the adverse effects of testosterone deficiency on the cardiovascular system start in the antenatal period. Hypospadias may be an independent risk factor for cardiovascular disease in males.

6.4. Growth, puberty and testicular function in boys born small for gestational age with a nonspecific disorder of sex development

Tack LJW, van der Straaten S, Riedl S, Springer A, Holterhus PM, Hornig NC, Kolesinska Z, Niedziela M, Baronio F, Balsamo A, Hannema SE, Nordenström A, Poyrazoglu S, Darendeliler FF, Grinspon R, Rey R, Aljuraibah F, Bryce J, Ahmed F, Tadokoro-Cuccaro R, Hughes I, Guaragna-Filho G, Maciel-Guerra AT, Guerra-Junior G, Cools M

Clin Endocrinol (Oxf). 2022 Feb;96(2):165-174.
PMID: 34668586, DOI: 10.1111/cen.14614.

Brief Summary: This retrospective case-control study used data from the international DSD registry to investigate the long-term outcomes of males born small for gestational age (SGA) with hypospadias/DSD. A large cohort of 179 boys (115 males born SGA; 64 appropriate for gestational age) was investigated for growth, pubertal development, and gonadal functions (serum LH, FSH, testosterone, AMH, and inhibin B levels) at minipuberty and puberty.

There is a significant association between being born small for gestational age (SGA) and having hypospadias or a more complex phenotype of DSD. Vice versa, boys with non-specific DSD were found to have low birth weight. Furthermore, there is some evidence that infants born with SGA have an increased risk of developing metabolic syndrome, lack of catch-up growth, and experiencing early puberty.

The results of this study reveal that boys with non-specific DSD who were born SGA are at higher risk of poor long-term growth outcomes than SGA boys without non-specific DSD. These boys may also have an altered gonadal function in early postnatal life although pubertal hormone levels seem unaffected. Fertility outcomes of SGA boys with non-specific DSD need further investigation.

6.5. Broad-spectrum XX and XY gonadal dysgenesis in patients with a homozygous L193S variant in *PPP2R3C*

Cicek D, Warr N, Yesil G, Kocak Eker H, Bas F, Poyrazoglu S, Darendeliler F, Direk G, Hatipoglu N, Eltan M, Yavas Abali Z, Gurpinar Tosun B, Kaygusuz SB, Seven Menevse T, Helvacioglu D, Turan S, Bereket A, Reeves R, Simon M, Mackenzie M, Teboul L, Greenfield A, Guran T

Eur J Endocrinol. 2021 Dec 1;186(1):65-72.
PMID: 34714774, DOI: 10.1530/EJE-21-0910.

Brief Summary: This brief report describes a novel gene, *PPP2R3C*, in the pathogenesis of complete and partial XY and XX gonadal dysgenesis (GD).

GD is a very rare condition with an estimated prevalence of 1–9 cases per 100,000 live-births. GD can be classified as either complete (CGD) or partial (PGD) depending on the clinical characteristics and gonadal morphology. A subgroup of patients with GD have additional syndromic features. So far, there are a small number of genes described in the etiology of syndromic GD including *RSPO1, LARS2, HSD17B4, HARS2, TWNK, ERAL1,* and *CLPP* in 46,XX, and *ARX, ATRX, DHH, GATA4, HHAT, SOX9, WT1* and *ZFPM2* in 46,XY patients. In 2019, *PPP2R3C* was included in the list of genetic causes of syndromic CGD in 46,XY, namely MEGD syndrome (Myo-Ectodermo-Gonadal Dysgenesis) (1). Besides XY-CGD, a number of extragonadal syndromic features, including typical facial gestalt, low birth weight, myopathy, rod and cone dystrophy, anal atresia, omphalocele, sensorineural hearing loss, dry and scaly skin, skeletal abnormalities, renal agenesis, and neuromotor delay characterize MEGD syndrome.

This brief report describes a homozygous c.578T>C (p.L193S) *PPP2R3C* variant identified in four patients from three unrelated families; one 46,XX girl with primary gonadal insufficiency, two girls with 46,XY CGD, and one undervirilised boy with 46,XY PGD. The developmental role of *Ppp2r3c* in mice (C57BL6/N) was explored using CRISPR/Cas9 genome editing. *Ppp2r3c* expression was identified in the majority of gonadal cell lineages, including Tcf21+ gonadal progenitors and Sox9+ and Fst+ supporting cells in XY and XX gonads, respectively. There was no evidence of sexual dimorphism in levels of expression. Low-level, widespread expression of *Ppp2r3c* during this period in a number of cell lineages that contribute to the gonadal supporting cells was consistent with a sex-determining function in mice. Heterozygous Ppp2r3c knockout mice appeared overtly normal and fertile. Inspection of homozygous embryos revealed evidence of dead embryos at various days post coitum which suggests that loss of function of *Ppp2r3c* is not compatible with viability in mice.

This report illustrates the association of *PPP2R3C* variants with GD of various severities both in 46,XX, and 46,XY individuals suggesting the critical role of *PPP2R3C* in human gonadal development. In the mouse model, *Ppp2r3c* is important for sex determination and has an essential role in development.

Reference

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6.6. Expanding DSD phenotypes associated with variants in the DEAH-box RNA helicase *DHX37*

Zidoune H, Martinerie L, Tan DS, Askari M, Rezgoune D, Ladjouze A, Boukri A, Benelmadani Y, Sifi K, Abadi N, Satta D, Rastari M, Seresht-Ahmadi M, Bignon-Topalovic J, Mazen I, Leger J, Simon D, Brauner R, Totonchi M, Jauch R, Bashamboo A, McElreavey K

Sex Dev. 2021;15(4):244-252.
PMID: 34293745, DOI: 10.1159/000515924.

Brief Summary: This genetic study provides data of a large cohort of 140 patients with DSD who were screened for *DHX37* variants.

DHX37 emerges as a frequent cause of nonsyndromic 46,XY gonadal dysgenesis, and 46,XY testicular regression syndrome. Since the first description of the gene (1), *DHX37* mutations were identified in 8-25% of some other groups of DSD patients (2,3). *DHX37* encodes an RNA helicase protein (DEAH-box helicase 37) and its mutations impair ribosome biogenesis. Defects in ribosome assembly can cause a wide range of defined human diseases in specific cell types. *DHX37* is one of the most highly conserved genes in the human genome and is intolerant to loss-of-function and missense variants in the general population. The mechanism, whereby a highly conserved ribosomal subunit can generate a specific "ribosomopathy" is unknown.

The authors identified 7 patients with *DHX37* variants out of 140 patients with DSD (5% of the cohort). The spectrum of phenotypes ranged from a 46,XY female with complete gonadal dysgenesis to unilateral testicular regression in a fertile male. Unlike all previously reported heterozygous pathogenic *DHX37* variants associated with 46,XY DSD, a boy with testicular regression who carried a homozygous *DHX37* variant (c.C1430T) was identified.

Understanding precisely how *DHX37* mutations lead specifically to DSD will serve as a paradigm for other ribosomopathies. According to the authors hypothesis in this paper, 46,XY DSD caused by *DHX37* variants is the result of increased nucleolar stress which causes a rapid and transient rise in WNT signaling leading to β-catenin stabilization. Inappropriate activation of the canonical WNT/β-catenin pathway favors the ovarian development and increased pro-ovary WNT-signaling in the somatic XY gonadal progenitor cells may be sufficient to disrupt testis determination.

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6.7. MAP3K1 variant causes hyperactivation of Wnt4/β-catenin/FOXL2 signaling contributing to 46,XY disorders/differences of sex development

Chen H, Chen Q, Zhu Y, Yuan K, Li H, Zhang B, Jia Z, Zhou H, Fan M, Qiu Y, Zhuang Q, Lei Z, Li M, Huang W, Liang L, Yan Q, Wang C

Front Genet. 2022 Mar 3;13:736988.

PMID: 35309143, DOI: 10.3389/fgene.2022.736988.

Brief Summary: This molecular study highlights a novel mechanism of action of Mitogen-activated protein kinase kinase kinase 1 (MAP3K1) in the development of testicular dysgenesis.

MAP3K1 is one of the most common genes that has been identified to cause 46, XY DSD and variants are attributed to ~14-18% of the 46,XY DSD cases (1,2). It can present as complete or partial gonadal dysgenesis even within the same kindred, with clinical manifestations ranging from severe DSD to milder phenotypes such as hypospadias, cryptorchidism, and micropenis.

Functional studies of MAP3K1 variants have demonstrated a gain of function effect, causing increased phosphorylation of downstream targets resulting in decreased expression of SOX9, important for the development of the testis, and increased expression of β-catenin. These gene expression changes mimic the signaling pathway in ovarian development and thus result in abnormal testicular development.

These authors tested the pro-ovarian Wnt4/β-catenin/FOXL2 signalling pathway in two affected siblings with 46,XY DSD due to a novel missense c.556A > G/p.R186G variant in the *MAP3K1* gene. The healthy 46,XX

mother carried the same variant. These patients had normal female external genitalia and complete gonadal dysgenesis. One of the sisters had bilateral dysgerminoma. The authors have transiently transfected testicular teratoma cells (NT2/D1) and ovary-derived granulosa cells (KGN) with wild-type and *MAP3K1*^{R186G} variants and showed reduced binding of the *MAP3K1*^{R186G} variant to Ub. The lower ubiquitination level of *MAP3K1*^{R186G} caused increased stability and increased expression of MAP3K1^{R186G} protein. They have also shown higher expression and activity of *p38* and *GSK3β* which subsequently led to overactivity of the Wnt4/β-catenin pathway. *FOXL2* mRNA and protein expression in the mutant group was increased and *DMRT1* and *FGFR2* expression were decreased compared to the wild-type group.

Overall, this study increases our understanding of the mechanism of action of *MAP3K1* showing that the *MAP3K1*^{R186G} variant upregulated in vitro expression of genes associated with ovarian development (including *WNT4*, β-catenin-*CTNNB1*, and *FOXL2*) and downregulated the expression of testicular development-related genes (*FGFR2* and *DMRT1*).

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6.8. Targeting the non-coding genome for the diagnosis of disorders of sex development

Atlas G, Sreenivasan R, Sinclair A

Sex Dev. 2021;15(5-6):392-410.
PMID: 34634785, DOI: 10.1159/000519238.

Brief Summary: This review summarizes how non-coding genomic variants are identified and validated, and why non-coding defects found in DSD patients have to be considered when investigating the genetic etiology of DSD.

Accurate genetic diagnosis of 46,XY DSD patients remains crucial for early treatment and prediction of associated risks of malignant tumors. Genetic studies that aim to investigate the etiology of DSD have focussed mainly on the coding genome. Despite the availability of techniques, such as targeted gene panel sequencing, whole-exome sequencing, and microarray analysis, the current DSD diagnostic rate for 46,XY DSD is limited to ~40%, and the rest of these patients yet lack a specific genetic diagnosis. On the other hand, a wide variety of non-coding defects are found in DSD patients, suggesting that aberrations in non-coding genomic regions could account for many of the remaining cases.

Therefore, at a genomic level, it is necessary to define regulatory regions of gonadal genes that are disrupted in DSD patients, identify active enhancers within these regions and detect their target promoters. Validation experiments both in vitro and in vivo will provide evidence of the functionality of these enhancers. The tools and approach algorithm used for the identification of variants in non-coding genomic regions associated to DSD etiology and in vivo and in vitro validation methods of these variants and enhancers were proposed in Figure 1.

Using these tools several CNVs disrupting the regulatory regions of gonad development genes including *SOX9, SOX3, SOX8, NR0B1, GATA4, DMRT1, WT1, NR5A1, AR,* and *SRY* have been discovered in DSD patients, with the *SOX9* regulatory region being the most extensively studied. Several duplications and deletions far upstream of *SOX9* were identified using array comparative genomic hybridization (CGH) on genomic DNA from 46,XX and 46,XY DSD patients, respectively. The review provides an excellent clinical update on these functional regulatory elements of the non-coding genome described in the pathogenesis of DSD so far.

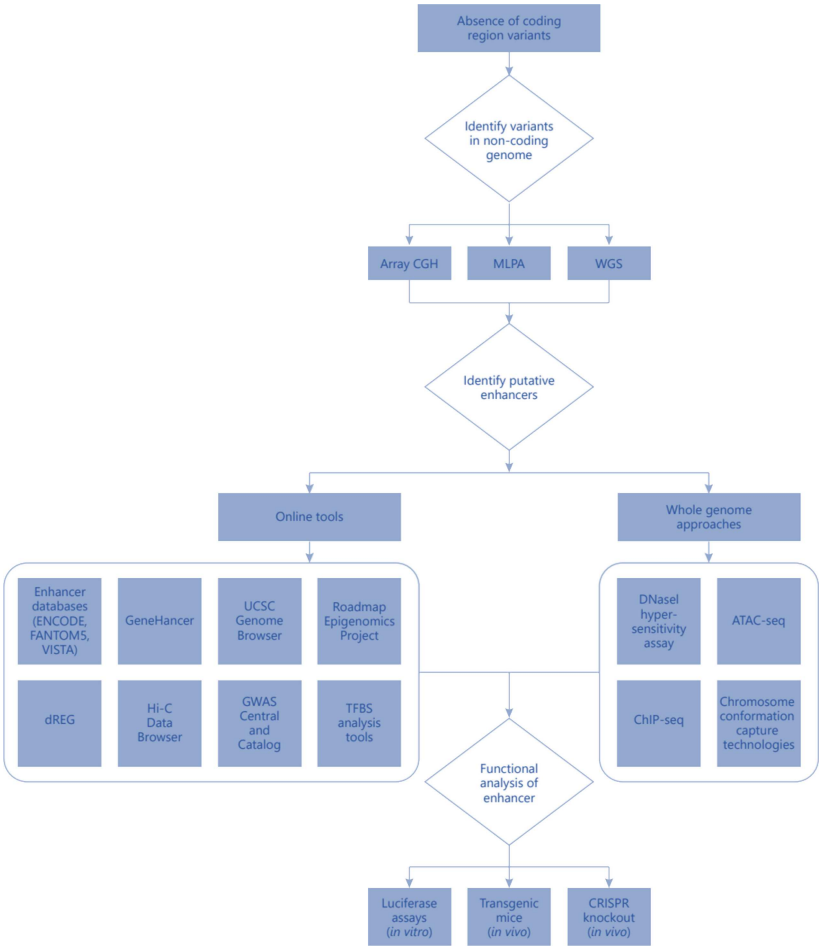

Figure 1. An approach to variant discovery in the non-coding genome for DSD patients. ATAC-seq, assay for transposase-accessible chromatin with high-throughput sequencing; CGH, comparative genomic hybridization; ChIP-seq, chromatin immunoprecipitation sequencing; CRISPR, clustered regulatory interspaced short palindromic repeat; dREG, discriminative regulatory element detection from GRO-seq; DSD, disorders of sex development; ENCODE, encyclopedia of DNA elements; FANTOM5, functional annotation of the mammalian genome project; GWAS, genome-wide association study; Hi-C, high throughput chromosome conformation capture; MLPA, multiplex ligation dependent probe amplification; TFBS, transcription factor binding site; UCSC, University of California Santa Cruz; WGS, whole genome sequencing.

6.9. Whole exome sequencing reveals copy number variants in individuals with disorders of sex development

Sreenivasan R, Bell K, van den Bergen J, Robevska G, Belluoccio D, Dahiya R, Leong GM, Dulon J, Touraine P, Tucker EJ, Ayers K, Sinclair A

Mol Cell Endocrinol. 2022 Apr 15;546:111570.

PMID: 35051551, DOI: 10.1016/j.mce.2022.111570.

Brief Summary: This report emphasizes the importance of screening for copy number variants (CNVs) using parallel genomic techniques for diagnosing unsolved cases of complete androgen insensitivity syndrome (CAIS) as well as other DSDs, where traditional sequencing techniques fail to detect a genetic cause.

The authors used a specific bioinformatics protocol (Ximmer pipeline) developed by Sadedin et al. (1) to detect CNVs using whole-exome sequencing (WES) data. They identified one 46,XY female patient with CAIS due to a rare duplication of exon 2 of the androgen receptor (*AR*) gene, and two siblings with primary ovarian failure (POI) due to homozygous deletion in *FSHR* and another POI patient with a heterozygous deletion in *NR5A1*. A specific molecular etiology was not identified in these 3 patients by targeted gene sequencing panel and whole-exome sequencing (WES) at initial investigations.

Androgen insensitivity syndrome (AIS) is a rare DSD resulting from variants of the X-linked *AR*. Patients with CAIS have a 46,XY karyotype and present with normal female external genitalia, bilateral intra-abdominal or inguinal testes, the absence of Müllerian structures, hypoplastic or absent Wolffian structures, primary amenorrhea, normal breast development, absent or sparse pubic hair. The prevalence of genetically confirmed CAIS ranges from 1:20400 to 1:99,100 46,XY individuals. Sanger sequencing or multi-gene massively parallel sequencing panels confirm the diagnosis of over 95% of CAIS patients. For cases where no variant is detected in AR, other technologies such as WES and array comparative genomic hybridization (CGH) may be used, with the former technique typically detecting single nucleotide variations (SNVs) and small insertions/deletions (INDELs) and the latter identifying copy number variations (CNVs). While whole-genome sequencing (WGS) may also be used to detect CNVs, it is currently not feasible for high throughput clinical diagnostics due to high costs.

Instead of targeted gene panel tests conducted concurrently with array CGH analysis for genetic diagnosis to detect SNVs, INDELs as well as CNVs, this study shows the utility of Ximmer bioinformatics pipeline by analyzing WES data as a single test, sufficient for this purpose. The use of this technique would save cost and time for both the patient and clinician. Furthermore, genomic testing offers a less invasive approach and could be routinely used for genetic diagnosis of CAIS patients in place of techniques that rely on the isolation of genital skin fibroblasts from the patient.

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6.10. Contribution of clinical and genetic approaches for diagnosing 209 index cases with 46, XY differences of sex development

Gomes NL, Batista RL, Nishi MY, Lerário AM, Silva TE, de Moraes Narcizo A, Benedetti AFF, de Assis Funari MF, Faria Junior JA, Moraes DR, Quintão LML, Montenegro LR, Ferrari MTM, Jorge AA, Arnhold IJP, Costa EMF, Domenice S, Mendonca BB
J Clin Endocrinol Metab. 2022 Apr 19;107(5):e1797-e1806.
PMID: 35134971, DOI: 10.1210/clinem/dgac064.

Brief Summary: This retrospective clinical research reports the clinical/biochemical, radiological, and genetic findings of a large cohort of 209 non-syndromic 46,XY DSD patients from a single tertiary center collected over the last 25 years in Brazil. A molecular diagnosis was achieved in 59.3% of patients.

The authors classified this cohort into 3 groups based on clinical, hormonal, imaging assessment, and histologic findings of the patients; i) gonadal dysgenesis, ii) disorders of androgen and anti-Mullerian hormone (AMH) secretion or action, and iii) DSD of unknown etiology. For the etiologic diagnosis, the patients were sequenced by Sanger, and/or massively parallel sequencing (MPS) technologies including DSD Target panel or whole-exome sequencing (WES).

Overall, a molecular diagnosis was achieved in 59.3% of the cohort. Pathogenic *NR5A1* variants were the most common genetic findings among patients initially classified as DSD of clinically unknown etiology, emphasizing its broad phenotype presentation. Deleterious *DHX37* variants were the most common genetic findings in patients with gonadal dysgenesis, especially in those with testicular regression syndrome.

The revised DSD diagnostic algorithm (1) still recommends clinical/biochemical and imaging testing as the initial approach for all individuals with a suspected DSD. Nevertheless, because of the overlapping

clinical/biochemical phenotypes, the authors recommend that MPS should be incorporated as a first-line approach because it potentially decreases the complexity of the diagnostic workup, would minimize patient handling, improve diagnosis accuracy, and probably decrease the costs.

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Controversies on the Timing of Genital Surgery in Individuals with DSD

6.11. The timing of genital surgery in somatic intersexuality: surveys of patients' preferences

Meyer-Bahlburg HFL

Horm Res Paediatr. 2022;95(1):12-20.

PMID: 35045418, DOI: 10.1159/000521958.

Brief Summary: This comprehensive review summarizes surveys of affected DSD patients' opinions on the timing of genital surgery published in the last 20 years.

The binary system of sex and gender has dominated human societies throughout history. In this regard, atypical secondary sex characteristics constitute a challenge to the binary system and are associated with variable degrees of psychosocial stigma. During the second half of the 20th century, with improved surgical techniques, even if usually not acutely medically necessary, "corrective" or "normalizing" genital surgery, in infancy and early childhood became widely accepted in Western societies, with the goals of reducing intersex-related stigma, minimizing parental anxiety, and facilitating gender development within the binary gender system. However, with the publication of examples of poor cosmetic and functional and unsatisfactory outcomes of early gender assignment and associated genital surgery, the timing of such surgery relative to the age of consent has become a highly controversial topic. Additionally, ethical concerns exist concerning elective medical procedures that are performed prior to the age of consent.

Overall, this review of surveys suggests that the movement against early genital surgery disregards the risk of the stigma associated with genital ambiguity at all stages of development and the wishes of most patients. Legislation that uniformly bans elective genital surgery in childhood is not based on scientific evidence and does not take into account the unique needs of each individual. The results of these surveys show that a case-specific individualized decision on the timing of surgery would be more appropriate.

Gender Incongruence: Growth and Fertility in Transgender Girls

6.12. Trans girls grow tall: adult height is unaffected by GnRH analogue and estradiol treatment

Boogers LS, Wiepjes CM, Klink DT, Hellinga I, van Trotsenburg ASP, den Heijer M, Hannema SE

J Clin Endocrinol Metab. 2022 Jun 6:dgac349.

PMID: 35666195, DOI: 10.1210/clinem/dgac349.

Brief Summary: This paper reports the adult height of 161 transgender girls (registered male at birth) from the Amsterdam Cohort of Gender Dysphoria. It finds no difference in adult height between use of standard and high estrogen doses.

There is currently very little information on the effects of GnRH analogue and gender affirming hormone treatments on growth and adult height in transgender adolescents. In this large patient cohort, 73 transgender girls were close to adult height at the start of treatment. Of those still growing, GnRHa treatment slowed height velocity, and the start of estradiol treatment as expected caused a growth acceleration. The population of the

Netherlands is amongst the tallest on the planet, and thus there are potentially more concerns around extreme tall stature in transgender women.

This study found no difference in the adult height achieved, in comparison with predicted or target heights. Furthermore, neither standard dose (2mg) nor high dose (6mg) estradiol treatment had a different outcome in this regard. Those patients who were historically given high dose ethinylestradiol had only a small reduction (3cm) in adult height. This particular treatment is no longer recommended in tall girls due to reduced fertility and potential longer term cancer risks and the authors state that transgender girls might be at similar risk.

6.13. Adolescent transgender females present impaired semen quality that is suitable for intracytoplasmic sperm injection even before initiating gender-affirming hormone treatment

Amir H, Perl L, Barda S, Lantsberg D, Becker AS, Israeli G, Azem F, Oren A. Ad Amir H, Perl L, Barda S, Lantsberg D, Becker AS, Israeli G, Azem F, Oren A
Reprod Sci. 2022 Jan;29(1):260-269.
PMID: 33788173, DOI: 10.1007/s43032-021-00561-y.

Brief Summary: This study from Israel investigated semen samples from 26 transgender girls aged 14-18 years and notes a general reduction in semen quality parameters.

Fertility counselling is mandatory in all transgender adolescents prior to considering hormone interventions, but in reality how successful is the process of fertility preservation? In this patient cohort of transgender girls, all except two had completed male puberty and all reproductive hormone parameters were within normal limits. A mean of three semen samples per person were collected. In comparison with WHO references, semen volume, sperm concentration, total sperm count and motility were all lower. There was a higher rate of abnormal sperm morphology also. The post cryopreservation thaw analysis showed that the samples were suitable only for intracytoplasmic sperm injection procedures and not conventional intrauterine insemination.

The authors explore potential reasons for this but did not find any direct associations. The young age of the cohort could be an explanation as semen quality improves with age. Around 25% were on psychotropic medications for depression and/or anxiety. Additionally, 28% practised 'tucking' of the genitalia. There were also suggestions for further research into biological mechanisms such as genetic variations in the androgen receptor and estrogen receptor ß. Low prenatal testosterone concentrations have been linked with gender incongruence and impaired spermatogenesis but this concept requires more exploration.

Discussions with young transgender adolescents on their options of creating a family in the future are challenging. However, even if fertility preservation is undertaken, the resulting outcomes may not always be successful.

Gender Incongruence: Wider Impacts of Gender Affirming Hormone (GAH) Treatments

6.14. Sex hormones drive changes in lipoprotein metabolism

Robinson GA, Peng J, Peckham H, Radziszewska A, Butler G, Pineda-Torra I, Jury EC, Ciurtin C
iScience. 2021 Oct 11;24(11):103257.
PMID: 34761181, DOI: 10.1016/j.isci.2021.103257.

Brief Summary: The authors explored the relationship between sex hormones and lipids in pre-pubertal children, young post-pubertal cisgender men and women, and transgender adolescents on gender affirming hormone (GAH) treatments using serum metabolomics to assess 149 lipids. High-density lipoproteins (HDL, typically atheroprotective) were higher and very-low- and low-density lipoproteins (typically atherogenic) were lower in post-pubertal cisgender women compared with cisgender men. There were no differences seen pre-puberty.

Knowing what happens to health outcomes from sex hormone treatments is important as there are sex differences in long-term cardiovascular disease (CVD) risk. GAH treatment in transgender adolescents induced

reversals of lipoprotein profiles, concluding that sex hormones regulate lipid metabolism *in vivo*. Thus, estradiol drives a typically atheroprotective lipid profile through upregulation of apolipoprotein (Apo)A1 expressing lipoproteins (HDL), which could contribute to the sexual dimorphism observed in CVD risk post puberty. In clinical management terms, if GAH is prescribed, this worsens CVD risk for transgender men, but likely improves CVD risk for transgender women.

In another smaller study, smaller changes in VLDL and ApoB were seen after testosterone treatment [1]. Together, this could inform sex-specific therapeutic strategies for CVD risk management, especially diet and lifestyle in transgender adolescents, and these are reviewed in Kean et al [2].

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6.15. Self-perception of transgender adolescents after gender-affirming treatment: a follow-up study into young adulthood

Arnoldussen M, van der Miesen AIR, Elzinga WS, Alberse AE, Popma A, Steensma TD, de Vries ALC

LGBT Health. 2022 May-Jun;9(4):238-246.

PMID:35475663, DOI: 10.1089/lgbt.2020.0494.

Brief Summary: This study reports changes in wellbeing and quality of life in 70 adolescents (49 transgender men and 21 transgender women) from the Amsterdam Center for Expertise on Gender Dysphoria. It finds evidence of improvements in functioning on GAH.

The purpose of gender-affirming hormone (GAH) treatments, and if chosen gender affirming surgery, for transgender adolescents is to improve wellbeing and quality of life and reduce the dysphoria-associated distress. In this cohort, self-perception was assessed before the start of GAH treatment (mean 14.65 years, SD 2.08) and at least 6 months after gender-affirming surgeries (mean 20.70 years, SD 1.49) by the Self-Perception Profile for Adolescents (SPPA), which is a self-report measure that examines self-perception on seven different domains: scholastic competence, social acceptance, athletic competence, physical appearance, behavioural conduct, close friendship, and global self-worth. The domains of physical appearance and global self-worth improved over the course of treatment. No domain worsened. Scholastic competence, social acceptance, athletic competence, and close friendship remained stable over time.

Thus we are now beginning to see objective evidence of the physical intervention pathways. Other population surveys are also pointing in the same direction suggesting that GAH treatment reduce depression and suicidality [1]. However, the mechanisms are unclear. In another study, Grannis et al. found increased amygdala-prefrontal cortex connectivity using functional MRI (fMRI) scanning in 19 testosterone treated compared with 23 untreated transgender adolescents, and noted lower anxiety and depression indices, and improved body satisfaction on treatment in a battery of self-report measures [2]. Although changes were seen on fMRI scans, it was impossible to infer a direct assoiciation between specific patterns of cerebral activity and GAH treatment.

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6.16. Mortality trends over five decades in adult transgender people receiving hormone treatment: a report from the Amsterdam cohort of gender dysphoria

de Blok CJ, Wiepjes CM, van Velzen DM, Staphorsius AS, Nota NM, Gooren LJ, Kreukels BP, den Heijer M

Lancet Diabetes Endocrinol. 2021 Oct;9(10):663-670.

PMID: 34481559, DOI: 10.1016/S2213-8587(21)00185-6.

Brief Summary: This paper reports standardised mortality rates (SMR) between 1972 and 2018 in a large cohort of 2927 transgender women and 1641 transgender men from the Amsterdam Cohort of Gender Dysphoria. It finds a higher SMR for transgender women compared with transgender men.

As paediatricians, we should be aware of the long-term outcomes of our interventions. All of the current cohort started gender-affirming hormone (GAH) when aged > 17 years and none received GnRHa. The median follow-up time was 11 years (IQR 4–22) for transgender women and 5 years (2–17) for transgender men, giving a total follow-up time of 40,232 person-years for transgender women and 17,285 person-years for transgender men. No decreasing trend in mortality risk was observed over time.

317 (10·8%) transgender women died, higher than expected in comparison with the general population of cisgender men (SMR 1·8, 95% CI 1·6–2·0) and the general population of cisgender women (SMR 2·8, 2·5–3·1). Cause-specific mortality in transgender women was high for cardiovascular disease, lung cancer, HIV-related disease, and suicide. In transgender men, 44 (2·7%) died, which is also higher compared with the general population of cisgender women (SMR 1·8, 95% CI 1·3–2·4) but not when compared with the general population of cisgender men (SMR 1·2, 95% CI 0·9–1·6). Cause-specific death in transgender men, although fewer in total number than in transgender women, had a higher SMR high for non-natural causes of death other than suicide. There was no direct association found between GAH treatment types and the increased mortality.

These findings highlight the need for good general health advice and support for transgender people, and also reaffirm the need to study the long-term outcomes of GAH in transgender adolescents.

7. Puberty

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Introduction

This year brought us some fascinating data regarding extra-hypothalamic GnRH neurons as well as a new player in puberty and growth regulation, *MC3R*. It also highlighted the crucial role of glial cells and tanycytes in the developmental processes leading to puberty. From a clinical point of view, this year's studies opened new avenues for better discriminating between self-limiting delayed puberty and isolated hypogonadotropic hypogonadism.

Clinical Guidance

7.1. Whole exome sequencing identifies deleterious rare variants in *CCDC141* in familial self-limited delayed puberty

Saengkaew T, Ruiz-Babot G, David A, Mancini A, Mariniello K, Cabrera CP, Barnes MR, Dunkel L, Guasti L, Howard SR
NPJ Genom Med. 2021 Dec 20;6(1):107.
doi: 10.1038/s41525-021-00274-w. PMID: 34930920
https://www.nature.com/articles/s41525-021-00274-w

Brief Summary: This cross-sectional study describes the genetic data of a cohort of patients with self-limited delayed puberty, finding a high prevalence of *CCDC141* gene mutations. Using *in silico* and cellular models, the authors identified a role for *CCDC141* mutants in delayed puberty likely caused by abnormal cell migration.

Self-limited delayed puberty (SLDP) is the most common cause of delayed puberty (DP)[1][2]. A family history of DP is frequently reported, with an autosomal dominant transmission and variable penetrance [3]. Only a small number of genes have been identified and are mostly associated with the GnRH network. This study evaluated the genetic data of a cohort of familial SLDP patients and explored the pathophysiological basis of identified mutations.

Whole exome sequencing data from 193 individuals (100 families) were analyzed. Relatives were included and categorized as affected, unaffected, or unknown. The 35 unaffected family members were used as controls. Five predicted deleterious variants in *CCDC141* were identified in 21 individuals (6% of the cohort). Mutations in this gene have been previously described in Kallman syndrome and Isolated Hypogonadotropic Hypogonadism (IHH) [4][5]. *CCDC141* is expressed in GnRH migrating neurons and olfactory axons during embryonic development and plays a crucial role in neuronal migration [4][5]. A homology modeling predicted that all variants were deleterious. *CCDC141* is known to play a role in the centrosome activity during the migration process [6]. *CCDC141* mutants showed abnormal subcellular localization associated with abnormal distribution of acetylated tubulin [6]. Mutant expression led to delayed migration in transfected HEK292.

In conclusion, this study described *CCDC141* mutations in SLDP patients for the first time. *CCDC141* mutations could lead to abnormal GnRH neuronal migration and lead to a phenotypic spectrum of SLDP or IHH depending on the severity of the defect. This study underlines the importance of embryonic development and cell migration in completing pubertal development.

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7.2. Genetic evaluation supports differential diagnosis in adolescent patients with delayed puberty

Saengkaew T, Patel HR, Banerjee K, Butler G, Dattani MT, McGuigan M, Storr HL, Willemsen RH, Dunkel L, Howard SR
Eur J Endocrinol. 2021 Oct 8;185(5):617-627.
doi: 10.1530/EJE-21-0387. PMID: 34403359.
https://eje.bioscientifica.com/view/journals/eje/185/5/EJE-21-0387.xml

Brief Summary: This study investigates the role of Whole Exome Sequencing in the differential diagnosis of delayed puberty, evaluating a genotype-phenotype correlation.

Delayed puberty (DP) comprises a spectrum of disorders ranging from isolated hypogonadotropic hypogonadism (IHH) to self-limiting delayed puberty (SLDP). The differential diagnosis between these conditions is difficult and no available investigation can reliably distinguish between them [2,3].

This study investigated the impact of genetic analysis on the diagnosis of DP in a cohort of 46 patients (40 males and 6 females). After clinical diagnosis of SLDP or IHH, subjects were followed until 18 years of age to confirm diagnosis. 25 patients were diagnosed as having SLDP, while 21 had IHH. Whole-Exome-Sequencing was performed in these patients and 35 controls, and results were filtered using a virtual panel of 47 known genes. Genotypic diagnosis was defined as SLDP, IHH or inconclusive. Potentially deleterious variants of 12 genes were found in 15 patients and a genetic diagnosis was possible for 1 SLDP and 7 IHH. In all cases, the genetic diagnosis fully corresponded to final clinical diagnosis. Three patients with initial SLDP diagnosis received a genetic diagnosis of IHH that was clinically confirmed at the end of follow-up. Clinical presentations for known pathogenic mutations corresponded to literature data (homozygous *TAC3* or *GNRHR*)[4,5]. Authors identified heterozygous variants in IHH causative genes (*DMXL2*, *OTUD4*, *SEMA3E*) in 3 patients with a final clinical diagnosis of SLDP. This indicates the potential overlap of pathophysiological mechanisms between SLDP and IHH. In this study, genetic analysis showed a 100% specificity and positive predictive value for the diagnosis of IHH. However, its sensitivity (33.3%) and negative predictive value (64.1%), were lower than other biochemical profilings, such as basal and stimulated gonadotropins, or basal inhibin B [3][6][7]. Further studies will be necessary to enrich these virtual panels and improve knowledge regarding these conditions.

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7.3. FSH-stimulated inhibin b (FSH-iB): a novel marker for the accurate prediction of pubertal outcome in delayed puberty

Chaudhary S, Walia R, Bhansali A, Dayal D, Sachdeva N, Singh T, Bhadada SK

J Clin Endocrinol Metab. 2021 Aug 18;106(9):e3495-e3505.
doi: 10.1210/clinem/dgab357. PMID: 34010394
https://academic.oup.com/jcem/article-abstract/106/9/e3495/6278376

Brief Summary: This prospective interventional study evaluates the stimulation of inhibin B by exogenous FSH as a promising predictor of spontaneous puberty onset.

The diagnostic approach to delayed puberty (DP) is still difficult and differential diagnosis between self-limited DP and Isolated Hypogonadotropic Hypogonadism (IHH) remains a major challenge. Recently, inhibin B levels seemed promising, but diagnostic thresholds are variable and overlapping [3]–[6]. The main stimulus to inhibin B is FSH, but inhibin B stimulation as a diagnostic tool had not been studied so far [7]. This study evaluate inhibin B stimulation by exogenous FSH as a predictor for puberty onset.

Two cohorts were studied: an exploratory cohort of healthy pubertal subjects (Group 1- 18 M/8 F) or IHH patients (Group 2 - 8 M/8 F), and a validation cohort of 19 subjects (11 M/8 F) followed for DP. To confirm the diagnosis, the validation group had been followed up until age 18 years. All subjects underwent an FSH stimulation test and a triptorelin stimulation test. In the exploratory cohort, Group 1 showed higher levels of basal inhibin B (219 pg/ml M - 100.35 pg/ml F) than Group 2 (29.32 pg/ml M vs 36.38 pg/ml F). This was confirmed and amplified for stimulated-inhibin B (FSH-ib) (408 pg/ml in Group 1 M vs 45.96 pg/ml in Group 2 M; 1165.75 pg/ml in Group 1 F vs 45.16 pg/ml in Group 2 F). A cut-off value of FSH-ib of 116.14 pg/mL in M and 116.50 pg/mL in F showed 100% sensitivity and specificity to identify puberty onset. FSHib was superior to baseline and stimulated LH levels (54.5%-87.5% diagnostic accuracy) and baseline inhibin B (81.8% - 87.5% diagnostic accuracy).

This study, although conducted in a limited sample size, reveals that inhibin B can be stimulated by exogenous FSH only in subjects with a functioning hypothalamic-pituitary-gonadal axis, making this a promising tool for diagnostic purposes. This test, compared to combined GnRH-analogue- and hCG or kisspeptin stimulation tests [8][9], requires fewer injections, shorter duration and no hospitalization.

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7.4. The use of morning urinary gonadotropins and sex hormones in the management of early puberty in chinese girls

Zhan S, Huang K, Wu W, Zhang D, Liu A, Dorazio RM, Shi J, Ullah R, Zhang L, Wang J, Dong G, Ni Y, Fu J

J Clin Endocrinol Metab. 2021 Oct 21;106(11):e4520-e4530.
doi: 10.1210/clinem/dgab448.
Erratum in: *J Clin Endocrinol Metab.* 2021 Nov 15;: PMID: 34160619
https://academic.oup.com/jcem/article/106/11/e4520/6308284

Brief Summary: This cross-sectional study of 355 girls with Central Precocious Puberty (CPP) evaluates the diagnostic role of urinary gonadotropins.

The gold standard diagnostic for CPP is the GnRH stimulation test (GnRH-ST), which is invasive and costly [1]. Previous studies described the detectability of urinary gonadotropins and hypothesized that First Morning Voided (FMV) urinary gonadotropins may play a role in assessing CPP [2], [3]. This study validated urinary gonadotropins in the diagnosis and monitoring of CPP.

The cross-sectional study included 355 girls with CPP aged between 3.9 and 9.8 years. GnRH-ST was used to categorize girls as having a positive (LH peak $>$ 5 IU/L, 258 girls) or negative (LH peak $<$ 5 IU/L, 97 girls) LH response. One FMV urine specimen was collected the same day. Using Pearson's correlation, uLH and uLH-to-uFSH ratio were strongly correlated with basal and stimulated serum gonadotropins. Using ROC analysis, authors defined a uLH cutoff value of 0.55 IU/L for the CPP screening, with a 95% sensitivity and a value of 1.74 IU/L for CPP diagnosis, with a 69.4% sensitivity and a 75.3% specificity to predict a positive GnRH-ST. When combining uLH with uLH-to-uFSH ratio $>$ 0.4 IU/L, the specificity increased to 86.6%. 20 patients receiving GnRH-analogue treatment were evaluated at 3 and 6 months of follow up and both serum gonadotropins and uLH indicated adequate suppression of the gonadal axis.

In conclusion, these findings show that FMV uLH may be an interesting alternative for CPP screening, but further studies will be necessary in independent cohorts to validate the method particularly in pre-pubertal and pubertal controls.

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7.5. Analysis of early-life growth and age at pubertal onset in US children

Aris IM, Perng W, Dabelea D, Ganiban JM, Liu C, Marceau K, Robertson OC, Hockett CW, Mihalopoulos NL, Kong X, Herting MM, O'Shea TM, Jensen ET, Hivert MF, Oken E

Program Collaborators for Environmental Influences on Child Health Outcomes

JAMA Netw Open. 2022 Feb 1;5(2):e2146873.

doi: 10.1001/jamanetworkopen.2021.46873. PMID: 35119461

https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2788643/

Brief Summary: This study describes the correlation between growth patterns during the first 5 years of life and onset of puberty. A sex-specific association was found between rapid weight and height gains and early onset of puberty.

A secular trend in timing of pubertal onset has been reported world-wide [1]. The mechanism is likely multifactorial. Some studies have hypothesized a role for altered growth patterns in childhood caused by environmental exposure or nutritional excess [2]–[5]. This prospective multi-cohort study evaluated the correlation between growth patterns during the first 5 years of life and the onset of puberty. 7495 children were included (3772 girls and 3712 boys) and examined during early infancy (first 5 months of life), late infancy (5 month – 2 years) and early childhood (2-5 years). The primary outcome was age at peak height velocity (APHV), while the secondary outcome was self-reported or parent-reported age at pubertal onset and age at menarche. Anamnestic data about pregnancy, maternal data, socioeconomic level, ethnicity and environmental exposure were collected. Girls had an earlier APHV than boys (10.8 vs 12.9 years). Boys with faster gain in weight, length or height or BMI exhibited earlier pubertal onset evidenced by earlier APHV. Girls with faster gains in weight and length or height in early childhood had earlier APHV, while girls with faster gains in weight and BMI in late infancy and early childhood exhibited earlier time at menarche and pubarche, respectively. In all cases, data were normalized for available anamnestic parameters. Comorbidities were not evaluated.

Although puberty was indirectly evaluated, this large multi-cohort study identifies rapid growth patterns during the first years of life as a potential risk factor for earlier pubertal onset.

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7.6. Gonadotropin-releasing hormone analogs for treatment of central precocious puberty in children less than 2 years of age

Gohil A, Eugster EA

J Pediatr. 2021 Dec 20:S0022-3476(21)01236-1.
doi: 10.1016/j.jpeds.2021.12.030. PMID: 34942182
https://www.sciencedirect.com/science/article/pii/S0022347621012361

Brief Summary: This study evaluates the efficacy and safety of GnRH analogue treatment with Central Precocious Puberty (CPP) for patients under 2 years of age. The authors conclude that GnRH analogue treatment is safe and effective in this age group.

GnRH analogues (GnRHa) represent the treatment of choice for CPP [1] and have been approved by the Food and Drug Administration for children aged 2 years and above. Although rare, CPP also occurs in children $<$ 2-year-old [2]–[4]. In the absence of any alternative, this situation requires off-label drug use which requires individual allowance and/or lack of coverage by health insurances that both may lead to delaying treatment in some countries. This study evaluated the safety and efficacy of GnRHa treatment in children $<$ 2-year-old.

43 subjects were identified in the literature and 4 of them from a retrospective evaluation at the Riley Hospital for Children. 56.3% were female, while 43.7% were male with an average age at start of GnRHa treatment of 14.5 ± 4.7 months. The cause of the CPP was hypothalamic hamartoma in 24 cases, Rathke cleft cyst in 2 cases, septo-optic dysplasia in 1 case and idiopathic-CPP in 5 cases. Their treatments included depot injections, histrelin implant and rapid-acting formulation. The treatment resulted in suppression of the Hypothalamic-Pituitary-Gonadal axis, with stabilization or regression of pubertal development, growth velocity and hormonal parameters in all cases.

The authors focused mostly on the short-term efficacy of the treatment, which was as effective as in other age groups. No adverse events were reported. Long-term efficacy, including parameters such as age of menarche or final height, was not analyzed. The authors found an equal gender distribution and a preponderance of organic causes [5]–[7], suggesting very different characteristics for CPP in infants and toddlers in comparison to CPP in children between 3-8 years.

In conclusion, age should not be a limiting factor for GnRHa treatment as its efficacy and safety in children aged less than 2 years seems comparable to that in older children.

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7.7. Connecting nutritional deprivation and pubertal inhibition via GRK2-mediated repression of kisspeptin actions in GnRH neurons

Perdices-Lopez C, Avendaño MS, Barroso A, Gaytán F, Ruiz-Pino F, Vázquez MJ, Leon S, Song YB, Sobrino V, Heras V, Romero-Ruiz A, Roa J, Mayor F Jr, Murga C, Pinilla L, Kaiser UB, Tena-Sempere M.

Metabolism. 2022 Apr;129:155141.

doi: 10.1016/j.metabol.2022.155141. Epub 2022 Jan 22. PMID: 35074314.

https://www.metabolismjournal.com/article/S0026-0495(22)00019-1/fulltext

Brief Summary: the authors used pharmacological and transgenic models to demonstrate the role of GRK2, G protein coupled-receptor kinase-2, as a metabolic modulator of kisspeptin action in GnRH neurons

The awakening of GnRH neurons at puberty is the result of an interplay between complex regulatory mechanisms [1], among which kisspeptin neurons play a master role [2]. While metabolic and nutritional factors are known to influence pubertal timing by affecting presynaptic compounds of kisspeptin signaling [3], postsynaptic regulation of kisspeptin action by metabolic factors also appear critical for puberty [4].

These authors investigated the role of G protein coupled-receptor kinase-2 (GRK2) in GnRH neurons as this GPCR is known to modulate kisspeptin receptor signaling *in vitro*[5]. *GRK2* expression decreased in the preoptic area after pubertal onset in female rats. Postnatal undernutrition induced an increase in hypothalamic *GRK2* expression and delayed puberty, and these effects were partially reversed by central administration of a GRK2 inhibitor. Moreover, this central inhibition led to advanced puberty in normally fed female rats and to a higher gonadotrophin response to kisspeptin. Finally, the authors used a transgenic mouse model of conditional ablation of *GRK2* in GnRH neurons and observed early onset of puberty, improved gonadotropin response to kisspeptins and increased LH pulse frequency. *GRK2* ablation partially prevented the negative impact of undernutrition on puberty onset and LH pulsatility.

Altogether, those *in vivo* experiments identify GRK2 as a novel modulator of kisspeptin action on GnRH neurons

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7.8. GnRH neurons recruit astrocytes in infancy to facilitate network integration and sexual maturation

Pellegrino G, Martin M, Allet C, Lhomme T, Geller S, Franssen D, Mansuy V, Manfredi-Lozano M, Coutteau-Robles A, Delli V, Rasika S, Mazur D, Loyens A, Tena-Sempere M, Siepmann J, Pralong FP, Ciofi P, Corfas G, Parent AS, Ojeda SR, Sharif A, Prevot V

Nat Neurosci. 2021 Dec;24(12):1660-1672.

doi: 10.1038/s41593-021-00960-z. Epub 2021 Nov 18. PMID: 34795451.

https://www.nature.com/articles/s41593-021-00960-z

Brief Summary: This neuroanatomical and physiological study in mice demonstrates that GnRH neurons attract glial cells in their vicinity via cell–cell communication during postnatal development, which is essential for proper reproductive function in adulthood.

The glial environment of GnRH neurons is essential for the regulation of GnRH release and reproductive function [1]. The mechanisms involved in the establishment of this glial microenvironment are still unknown.

Based on the evidence that in the neocortex astrocyte formation in the neocortex is regulated by extrinsic signals generated by neurons [2], the authors hypothesized that GnRH neurons could be responsible for the recruitment of glial cells in their vicinity during postnatal development. By mapping cell proliferation in the preoptic area during mouse postnatal development, they determined that the progenitors associated to GnRH neurons would mainly generate astrocytes. Blocking gliogenesis with an anti-mitotic led to delayed sexual maturation in female mice. Postnatal exposure to the endocrine disruptor bisphenol A similarly delayed puberty and disrupted the abililty of GnRH neurons to recruit progenitors. Using *in vitro* assays, the authors discovered that GnRH neurons responded to incoming glial signals by synthesizing prostaglandin D2 (PGD2). Inhibition of PGD2 receptor in the preoptic area during infantile period led to alterations of GnRH neuron firing, disruption of minipuberty and delayed acquisition of reproductive capacity in female mice.

This study reveals a new neuron-to-neural-progenitor communication process that is essential for the postnatal development of the glial environment of GnRH neurons and for proper reproductive function in female mice. Moreover, this process can be altered by EDC treatment and lead to disrupted puberty/reproduction.

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7.9. Sex-specific pubertal and metabolic regulation of Kiss1 neurons via Nhlh2

Leon S, Talbi R, McCarthy EA, Ferrari K, Fergani C, Naule L, Choi JH, Carroll RS, Kaiser UB, Aylwin CF, Lomniczi A, Navarro VM
Elife. 2021 Sep 8;10:e69765.
doi: 10.7554/eLife.69765. PMID: 34494548.
https://elifesciences.org/articles/69765

Brief Summary: Using a database for arcuate nucleus transcripts, this study identifies *Nhlhl2* as a key regulator of the *Kiss1* gene in male mice.

The timing of puberty onset is influenced by the metabolic state. The population of kisspeptin neurons in the arcuate nucleus (ARC) plays a key role in the transmission of metabolic signals, such as leptin or insulin, to GnRH neurons [1]. Many potential binding sites for transcriptional factors (TFs) have been described in the *Kiss1* promotor [2], but their role in the transmission of metabolic information has not been investigated.

These authors used a previously described database of ARC transcripts [3] and identified that *Nhlh2*, a transcriptional factor, is highly enriched in ARC kisspeptin neurons. Based on bioinformatic tools and *in vitro* assays, it appeared that *Kiss1* and *TAC3* (coding for Neurokinin b) genes possess a binding site for *Nhlh2* and can be activated by this TF. To determine whether *Nhlhl2* has a physiological role in kisspeptin neurons, the authors generated a *Kiss1*-specific *Nhlh2* knock-out (KO) mouse. Interestingly, KO males presented a more severe phenotype than KO females: their puberty was delayed and their LH response to fasting and leptin was impaired. *Nhlh2* had previously been identified as affecting GnRH network development with a sexual dimorphism: Whole-body *Nhlh2* KO male mice are infertile while whole-body *Nhlh2* KO female mice are hypogonadal with irregular cycles but still fertile [4].

Overall, this study sheds lights on regulatory mechanisms at the level of *Kiss1* promotor, especially in response to nutritional status, and highlights once again the importance to study both sexes.

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7.10. The cryptic gonadotropin-releasing hormone neuronal system of human basal ganglia

Skrapits K, Sárvári M, Farkas I, Göcz B, Takács S, Rumpler É, Váczi V, Vastagh C, Rácz G, Matolcsy A, Solymosi N, Póliska S, Tóth B, Erdélyi F, Szabó G, Culler MD, Allet C, Cotellessa L, Prévot V, Giacobini P, Hrabovszky E

Elife. 2021 Jun 15;10:e67714.

doi: 10.7554/eLife.67714. PMID: 34128468

https://elifesciences.org/articles/67714

Brief Summary: This histological and transcriptomic study evaluates the presence and the potential role of extra-hypothalamic GnRH neurons in humans and identifies cholinergic GnRH-synthesizing cells in the human basal ganglia and basal forebrain.

Hypothalamic GnRH neurons originate from the olfactory placodes and migrate through a ventral path to the hypothalamus, where they control reproduction [1]. Studies have identified a dorsal migration pathway of GnRH neurons to the pallial and/or subpallial brain structures [2],[3] as well as expression of GnRH mRNA/immune-reactivity in extra-hypothalamic regions not related to reproduction both in humans and non-human primates [4],[5].

This study confirmed the presence of extra-hypothalamic GnRH (eh-GNRH) neurons in the human brain and evaluated their function. The authors identified almost 150,000-200,000 eh-GnRH neurons located in the basal ganglia and forebrain of the human brain. The putamen (Pu) contains most of these cells (82%), while other regions included the nucleus accumbens, caudate nucleus (Cd) and nucleus basalis magnocellularis (nbM). These neurons secreted authentic GnRH decapeptide derived from the *GNRH1* transcript, while its metabolite GNRH1-5 was present at lower levels. They express choline acetyltransferase (ChAT). Surprisingly, the ChAT phenotype was also present in 34.6% of hypothalamic GnRH neurons, a phenomenon not observed in other species before. The authors also showed that GnRH neurons become cholinergic after entering the brain.

This fascinating study reveals the complexity of the GnRH system beyond its reproductive function and opens new avenues regarding the potential role of such GnRH neurons in neurodegenerative diseases affecting cholinergic circuits. Symptoms in Alzheimer's disease for instance are due to the loss of basal forebrain cholinergic neurons, some of which show GnRH immune-reactivity. Additionally, the whole-transcriptome analysis of cholinergic inter-neurons dissected from the putamen could identify some alternative pathways to counteract the hyperactivity of cholinergic neurons, which explains some treatment-resistant symptoms in Parkinson's disease.

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7.11. Selective depletion of adult GFAP-expressing tanycytes leads to hypogonadotropic hypogonadism in males

Butruille L, Batailler M, Cateau ML, Sharif A, Leysen V, Prévot V, Vaudin P, Pillon D, Migaud M

Front Endocrinol (Lausanne). 2022 Mar 16;13:869019.

doi: 10.3389/fendo.2022.869019. PMID: 35370973.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8966543/

Brief Summary: This mouse study explores the function of alpha-tanycytes, which are neurogenic stem cells located in the medio-basal hypothalamus and expressing GFAP. GFAP-cell deficiency leads to hypogonadotropic hypogonadism, demonstrating the role of tanycytes in the regulation of reproduction.

A neurogenic niche was recently identified in the medio-basal hypothalamus of humans, rodents and sheep [1], [2]. Radial glia-like cells lining the ventricular wall, namely tanycytes, were identified as the neural stem/progenitor cells (NSPCs) in this region [3]–[5] and express glial fibrillar acid protein (GFAP) [6], [7].

This study investigated the role of GFAP-positive tanycytes in physiological functions controlled by the hypothalamus. To explore the role of adult GFAP-expressing tanycytes in hypothalamic function, the authors used transgenic mice expressing Herpes Simplex Virus-Thymidine kinase under the control of the GFAP promoter and subjected them to intra-cerebroventricular infusion of Ganciclovir (GCV), that selectively eliminated GFAP-positive dividing cells. *In vitro*, this depletion markedly decreased the neurospherogenic capacities of this region, indicating that elimination of alpha-tanycytes completely halted cell proliferation. *In vivo*, suppression of GFAP-expressing tanycytes had no effect on body weight or food intake, while decreased testes weights and drastically reduced testosterone secretion by Leydig cells, associated with decreased circulating LH levels. This pattern is suggestive of hypogonadotropic hypogonadism, which was consistent with decreased GnRH immunoreactivity both in the pre-optic area and in the median eminence. Although Ganciclovir exposure itself is harmful to the testicle [8], [9], it generally occurs at higher doses and was not observed in the control group.

The existence of this neural stem cell niche indicates the presence of hypothalamic neuronal regeneration, that seems to control reproductive function and, as described in other studies, food intake [3]. This could have an important clinical implication and further studies will be needed to evaluate the possible role of these stem cells in the treatment of hypothalamic disease.

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7.12. *MC3R* links nutritional state to childhood growth and the timing of puberty

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, BochukovaEG, 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

Nature. 2021 Nov;599(7885):436-441.

doi: 10.1038/s41586-021-04088-9. Epub 2021 Nov 3. PMID: 34732894.

https://www.nature.com/articles/s41586-021-04088-9

Brief Summary: This combination of a multi-cohort and animal studies describes the clinical impact of *MC3R* gene mutations. The authors identified a clinical syndrome caused by *MC3R* deficiency associating pubertal

delay, short stature and low IGF1 levels, illustrating the role of *MC3R* in linking nutritional state to growth and puberty.

The melanocortin system controls food intake and energy expenditure, while its role in puberty is unknown [1]. *MC3R* encodes a receptor in the brain [2],[3] for which a role in growth and puberty has been hypothesized [4],[5]. This study characterizes the role of *MC3R* through the evaluation of functional impairment associated with naturally occurring mutations. 200,000 subjects of the UK Biobank study were analyzed by whole-exome-sequencing. *MC3R* loss-of-function mutations were associated with moderate pubertal delay, shorter stature and lower serum IGF1 levels, lower lean mass but no difference in BMI or body fat. Among 3 of the more common variants, 2 showed functional impairment evaluated by cAMP production after stimulation with α-MSH *in vitro* while one had normal function, but all were phenotypically associated with delayed puberty. Variants associated with *in vitro* loss-of-function (LoF) correlated with larger effects on puberty, height and lean mass. One subject with a rare homozygous LoF mutation had delayed puberty, normal fertility, markedly short stature, low sitting/standing height ratio, low IGF1 levels and severe obesity. Through RNA sequencing in mice, the authors found enriched *MC3R* expression in KNDy and GHRH neurons, and increasing expression during postnatal development. *MC3R*-deficient mice had delayed sexual maturation, demonstrating a conservation of *MC3R* function through species.

This study identified a new clinical syndrome caused by *MC3R* deficiency, characterized by delayed puberty, short stature and low IGF1 levels. Importantly, it strengthens the evidence for a link between pubertal timing and nutrtional status as observed in overweight or underweight children, and proposes a plausible causal association between greater caloric availability and the global trends towards taller human height and earlier onset of puberty, which have been documented for several decades.

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7.13. Multi- and transgenerational outcomes of an exposure to a mixture of endocrine-disrupting chemicals (EDCs) on puberty and maternal behavior in the female rat

López-Rodríguez D, Aylwin CF, Delli V, Sevrin E, Campanile M, Martin M, Franssen D, Gérard A, Blacher S, Tirelli E, Noël A, Lomniczi A, Parent AS

*Environ Health Perspect.*2021 Aug;129(8):87003.

doi: 10.1289/EHP8795. Epub 2021 Aug 12. PMID: 34383603.

https://ehp.niehs.nih.gov/doi/10.1289/EHP8795?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

Brief Summary: Using a rodent model of exposure to a mixture of endocrine disrupting chemicals (EDCs), the authors identified transgenerational disruption of sexual maturation via hypothalamic epigenetic reprogramming.

The secular trend in the onset of puberty appears to be explained at least in part by exogenous environmental factors [1]. Among them, EDCs have been largely reported to affect sexual maturation in rodents [2] by targeting its central hypothalamic regulation [3]. However, the consequences of EDC exposure on puberty across generations have been much less investigated.

These authors used a mixture of 13 estrogenic or anti-androgenic EDCs at doses relevant to human exposure [4]. Ancestral exposure to the mixture delayed pubertal onset and altered folliculogenesis and estrous cyclicity in F2 and F3 generations. The pubertal delay found in F3 EDC exposed females was associated with a delayed

maturation of GnRH secretion and involved epigenetic reprogramming of key hypothalamic genes involved in the control of puberty such as *Kiss1*. In addition, F1 to F3 maternal behavior was impaired and associated with a loss in hypothalamic dopaminergic signaling. As altered maternal behavior is known to affect pubertal timing of the descendants [5], the authors used a cross-fostering model and demonstrated that maternal phenotype was recovered in EDC-exposed pups raised by unexposed dams, while puberty was still delayed.

Overall, this study showed that rats developmentally exposed to an EDC mixture exhibited multi- and transgenerational disruption of sexual maturation and maternal care via hypothalamic epigenetic reprogramming. These results raise concerns about the impact of environmentally relevant EDC mixtures on future generations

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8. Adrenals

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Preface

For this year's chapter, we searched PubMed for articles on 'adrenal' or 'steroidogenesis' published in English between June 1, 2021 and May 31, 2022. Our search yielded more than 5,000 citations. We examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we have avoided topics that have been discussed in the Yearbook 2021, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) The developmental origin and the specification of the adrenal cortex in humans and cynomolgus monkeys; ii) Corticosterone induces discrete epigenetic signatures in the dorsal and ventral hippocampus that depend upon sex and genotype: focus on methylated *NR3C1* gene; iii) A multi-classifier system to identify and subtype congenital adrenal hyperplasia based on circulating steroid hormones; iv) Crinecerfont lowers elevated hormone markers in adults with 21-hydroxylase deficiency Congenital Adrenal Hyperplasia; and v) results from two phase 2 studies of Tildacerfont in adults with classic Congenital Adrenal Hyperplasia.

Mechanism of the Year: The Developmental Origin and Specification of the Adrenal Cortex

8.1. The developmental origin and the specification of the adrenal cortex in humans and cynomolgus monkeys

Cheng K, Seita Y, Moriwaki T, Noshiro K, Sakata Y, Hwang YS, Torigoe T, Saitou M, Tsuchiya H, Iwatani C, Hosaka M, Ohkouchi T, Watari H, Umazume T, Sasaki K

Sci Adv. 2022; 8(16): eabn8485.

PMID: 35442744

https://pubmed.ncbi.nlm.nih.gov/35442744/

Brief Summary: This histologic and transcriptomic study of human and primate adrenal tissue samples provides a molecular framework to understand the fetal development of the adrenal gland.

The adrenal cortex is the major source of steroid hormones that drive a plethora of critical physiologic functions. Accordingly, developmental abnormalities in the formation of the adrenal cortex lead to various congenital and adult-onset diseases. Mammalian gonads (ovaries and testes) also produce steroid hormones, and commonalities exist between the adrenal cortex and gonads in both their steroid synthetic pathways and developmental origin.

In humans, the gonads and the adrenal cortex are first recognized as morphologically distinct structures at approximately 33 days after conception [Carnegie stage (CS) 15], at which time the adrenal cortex is recognized as a condensed blastematous structure, medial to the mesonephros, referred to as the adrenal primordium (**1-3**). However, because of the lack of genetic tracing tools or appropriate markers to differentiate emerging gonadal and adrenocortical lineages, how and when these lineages are specified and segregated remain poorly understood in humans.

These authors provide evidence that, in contrast to mice, the adrenal cortex in humans and cynomolgus monkeys originates in a spatially, temporally, and phenotypically distinct manner from that of the gonads. Specifically, through histologic and transcriptomic analyses of early human gonadogenesis, they found that the gonad is established from the posterior coelomic epithelium (CE) at 4 to 5 weeks post-fertilization through the sequential activation of GATA4 and NR5A1, similar to mice and cynomolgus monkeys. The adrenal primordium arises from adrenogenic coelomic epithelium via an epithelial-to-mesenchymal transition, which then progresses into the steroidogenic fetal zone via both direct and indirect routes. They demonstrated that adrenocortical and gonadal lineages exhibit distinct HOX codes, suggesting distinct anterior-posterior regionalization. In summary, these findings reveal the distinct origin of the human and infrahuman primate adrenal cortex and gonads, and provide an example of the divergence of organ morphogenesis between species, essential insight for understanding human adrenogenesis and gonadogenesis, and a molecular framework for understanding human adrenal and gonadal disorders.

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New Mechanisms

8.2. Corticosterone induces discrete epigenetic signatures in the dorsal and ventral hippocampus that depend upon sex and genotype: focus on methylated *NR3C1* gene

Caradonna SG, Einhorn NR, Saudagar V, Khalil H, Petty GH, Lihagen A, LeFloch C, Lee FS, Akil H, Guidotti A, McEwen BS, Gatta E, Marrocco J

Transl Psychiatry. 2022; 12(1): 109.

PMID: 35296634

https://pubmed.ncbi.nlm.nih.gov/35296634/

Brief Summary: This mouse study identified sex and genotype-dependent effects of oral corticosterone on behavioral and physiological outcomes as well as on gene expression and epigenetics in hippocampal subregions.

Glucocorticoids exert their effects by binding to glucocorticoid receptors (GRs), which regulate up to ~20% of the genome via both direct (by binding to glucocorticoid responsive elements in promoter regions) and indirect mechanisms (by interacting with bound transcription factors and epigenetic modifiers) (**1, 2**). GRs also play a key role in the feedback regulation of the hypothalamic- pituitary-adrenal (HPA) axis. They are highly expressed in the hippocampus and their distribution is heterogeneous depending on the hippocampal subregion both at baseline and in response to stress (**3, 4**). The dorsal (dHPC) and the ventral hippocampus (vHPC) are two functionally distinct subregions that differ in their respective neuroanatomical connectivity and in the biological processes they encode. Regional differences in gene expression also contribute to distinguish the function of the dHPC and vHPC in response to environmental stimuli. These two hippocampal circuits also show sex differences in neuronal proliferation (**5, 6**), indicating that males and females use distinct networks to modulate the function of the HPA axis. However, little is known on the whole-genome profile that underlies sex differences in the dHPC and vHPC, especially in response to glucocorticoids.

These authors gave exogenous GCs (oral corticosterone) to mice that were either wild-type (WT) or heterozygous for the brain-derived neurotrophic factor (*BDNF*) Val66Met (hMet) allele, a variant associated with genetic susceptibility to stress. They studied the effects of oral corticosterone on genomic differences in the hippocampal subregions, dorsal (dHPC) or ventral (vHPC). Gene expression was analyzed using RNA-sequencing and epigenetic regulation was assessed using reverse transcriptase-quantitative polymerase chain reaction and methyl-DNA-immuno-precipitation assay. Behavioral and physiological responses to corticosterone were also assessed.

Male mice showed increased anxiety- and depression-like behavior, while females showed affective behavior in response to corticosterone. Male mice also showed lower glycemia. Gene expression responses to corticosterone were greater in the vHPC than in the dHPC in males of both genotypes, and larger in the vHPC of males compared to females regardless of genotype. Moreover, differentially expressed genes (DEGs) in the vPHC exhibited sex differences related to glucocorticoid receptor (GR)-binding genes and epigenetic modifiers. DEGs showed differences in expression in the vHPC of females and discordant corticosterone-induced DEGs in both brain regions only in hMet males and females. Interestingly, the pattern of gene expression after corticosterone exposure mirrored the behavioral sex differences, as well as the behavior found in hMet females. Finally, they found differential methylation of exons 1C and 1F of the GR gene (*Nr3c1*) in hMet females.

This is the first study reporting behavioral sex differences in mice given oral corticosterone, impairing the hypothalamic-pituitary-adrenal axis, as well as hippocampal region-specific genomic expression profiles. The results open new avenues for research regarding the effects of stress and/or GC treatment on mood disorders and behavior.

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8.3. DNA methylation signatures in human neonatal blood following maternal antenatal corticosteroid treatment

Kim B, Sasaki S, Murphy K, Matthews SG

Transl Psychiatry. 2022; 12(1): 132.

PMID: 35354798

https://pubmed.ncbi.nlm.nih.gov/35354798/

Brief Summary: This case control study identified widespread effects of fetal exposure to antenatal maternal glucocorticoid treatment on newborn bloodspot DNA methylation profiles.

Antenatal treatment with glucocorticoids (AGC), such as betamethasone or dexamethasone, is widely used in pregnancies at risk of preterm birth to promote fetal organ maturation and reduce perinatal morbidity. Epidemiological studies have identified associations between AGC exposure and increased risk for cardiometabolic, immune and neurodevelopmental disorders in the offspring (**1-3**). Altered DNA methylation (DNAm) that is sustained across cell divisions may represent a mechanism for mediating long-lasting phenotypic changes to environmental exposures. In a guinea pig model, the authors previously showed that betamethasone given antenatally altered the DNAm in the prefrontal cortex and hippocampus, and the differences in DNAm were associated with a more hyperactive phenotype. Four genes (*C9orf116*, *Calb1*, *Glra3*, and *Gpr52*) explained 20–29% of the observed variability in locomotor activity (**4**). In the current study, the

authors analyzed DNAm 24-h post-birth in whole blood (obtained from dried blood spots) from 14 term-born infants exposed to betamethasone during late gestation (GW 24-33; 12 mgx2, 24 h apart) and compared them with controls.

Reduced Representation Bisulfite Sequencing (RRBS) libraries were prepared from 100 ng of high-quality dsDNA. RRBS libraries were sequenced using the Illumina NextSeq500 platform.

In total, 505 differentially methylated sites (DMCs) were identified in human neonatal blood following AGC treatment ($\geq$5% methylation difference, FDR $<$ 0.05 for n = 14/treatment), of which 231 sites were hypermethylated, representing 46%, and 274 were hypomethylated (54%). 15 sites, all hypomethylated (−25.91 to −43.25%), were localized within one DNase-H3K4me3 region (EH38E1382446). Examined in context of all 505 DMCs, region EH38E1382446 was observed in proximity to two additional DNase-H3K4me3 regions (EH38E1382445, EH38E1382449) and one promoter (E1382450), which were all hypomethylated (−7.89 to −43.25%, avg −22.55%). 35 DMCs were identified in this region, spanning 1432 base- pairs (chr1: 147078133-147079565).

The authors annotated 74 genes to hypermethylated DMCs, 100 genes to hypomethylated sites, and 45 genes to regions where methylation changes occurred in both directions. Of the top ten differentially methylated genes (*HSPG2, USP48, CELA3B, SH3PXD2A, NTM, YEATS2, MCF2L2, CAMK2N2, MAP6D1, PKP3*), four genes (*HSPG2, USP48, CELA3B, NTM*) contained glucocorticoid response elements (GRE) within their promoter regions, and five genes (*USP48, SH3PXD2A, NTM, CAMK2N2, MAP6D1*) are known to be highly expressed in the brain and have neurological roles.

In summary, antenatal GC treatment results in changes in DNA methylation in newborn blood. The differentially methylated genes were enriched for transcription regulation. Hypomethylated genes were enriched for pathways of proteasome activity. A number of the genes identified to be differentially methylated in human blood were also previously identified as differentially methylated in blood and hippocampus in guinea pigs following AGC treatment and were enriched for pathways of neurodevelopment. These findings enhance our understanding of the biological events that occur in response to exposure to prenatal glucocorticoids, such as ACS or maternal stress during pregnancy. The peripheral biomarkers presented in this study may help to identify individuals who are most at risk of developing altered phenotypes and enable future studies to design targeted intervention strategies and therapies to prevent or ameliorate the effects following prenatal adversity.

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Important for Clinical Practice

8.4. A multi-classifier system to identify and subtype congenital adrenal hyperplasia based on circulating steroid hormones

Ye L, Zhao Z, Ren H, Wang W, Zhou W, Zheng S, Han R, Zhang J, Li H, Wan Z, Tang C, Sun S, Wang W, Ning G

J Clin Endocrinol Metab. 2022; dgac271.

PMID: 35512387

https://pubmed.ncbi.nlm.nih.gov/35512387/

Brief Summary: This study, in a series of independent patient cohorts, developed and validated a clinical score, based on the circulating concentrations of 13 major steroid hormones, to detect and subtype Congenital Adrenal Hyperplasia (CAH).

Steroidogenesis is a complex process that plays a pivotal role in numerous cellular/physiological functions (**1**). A total of 14 enzymes or cofactors are required for adrenal steroidogenesis, producing 16 major steroid

metabolites (**3**). Genetic deficiencies in steroidogenesis enzymes impair glucocorticoid biosynthesis, and lead to Congenital Adrenal Hyperplasia (CAH), which includes 9 subtypes (**2**). CAH is one of the most common autosomal recessive disorders. The presentation of CAH is extremely variable, mostly owing to the complexity of the steroidogenesis pathway and the numerous disorder subtypes (**1, 2**). Each steroidogenesis enzyme or cofactor catalyzes multiple reactions. Individual subtypes may show overlapping presentations. Moreover, as mutant enzyme activity can be either mildly affected or completely inactivated, a continuum of disease phenotypes have been reported, from potentially life-threatening to infertility, and in some cases, individuals are completely asymptomatic (**2**).

The diagnosis of CAH often includes a tedious and uncertain workup. For the most common form of CAH, 21α-hydroxylase deficiency (21OHD), an ACTH stimulation test is usually required to confirm the diagnosis, especially for non-classic 21OHD (**3, 4**). Diagnostic criteria are lacking for other forms of CAH, such as 17α-hydroxylase/17,20-lyase deficiency (17OHD) and 11β-hydroxylase deficiency (11βOHD), even when genetic data are available, and no guidelines or consensus statements have been published. These challenges may result in misdiagnosis or delayed diagnosis of CAH. Profiling of multiple steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in patients with steroid-hormone disorders has revealed distinctive steroid patterns associated with these disorders (**4-6**).

These authors describe a multi-classifier system to diagnose CAH by measuring a panel of 13 steroid hormone levels by LC-MS/MS in a single baseline blood sample. A cascade logistic regression model was performed to generate the "Steroidogenesis Score" to distinguish the three most common CAH subtypes 11βOHD, 17OHD and 21OHD in a discovery cohort (N = 226). This was then validated in an independent cohort (N = 111) and finally applied in a prospective cohort of 256 patients, where the Steroidogenesis Score showed high diagnostic accuracy: 11βOHD (AUC, 0.994), 17OHD (AUC, 0.993) and 21OHD (AUC, 0.979). For patients with non-classic 21OHD, the system had higher sensitivity than basal 17α-hydroxyprogesterone (17OHP) (AUC, 0.973 vs 0.840, p = 0.005) and was non-inferior to basal and ACTH-stimulated 17OHP (AUC, 0.973 vs 0.947, p = 0.681).

In summary, this biochemically steroidogenesis score showed high diagnostic accuracy, and will be especially useful for patients with the non-classic form of the disease. The test is easily conducted with a single blood draw. The use of data-learning approaches, such as the steroidogenesis score, may greatly increase the diagnostic accuracy and efficiency for this rare disease and possibly others.

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8.5. Adrenal steroids reference ranges in infancy determined by LC-MS/MS

Enver EO, Vatansever P, Guran O, Bilgin L, Boran P, Turan S, Haklar G, Bereket A, Guran T

Pediatr Res. 2021; 92(1):265-274.

doi: 10.1038/s41390-021-01739-5.

PMID: 34556810

https://pubmed.ncbi.nlm.nih.gov/34556810/

Brief Summary: This study provides a detailed set of normative reference values for steroidogenesis during the first 6 months of life, which may facilitate rapid testing of infants for steroidogenic disorders.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become a relevant technique for clinical diagnostics. It has high analytical sensitivity and can quantify several analytes simultaneously. In adrenal

enzyme deficiencies, accumulation of specific precursors occurs along with reduction of its relative steroid product. These disorders are often diagnosed in the first months of life and LC-MS/MS is, therefore, a useful methodology in order to assess steroidogenesis, because it provides a more complete picture using small sample volumes. However, normative data are required for valid interpretation.

These authors provide normative reference data for 14 adrenal steroids in the first 6 months of life, along with precursor-to-product steroid ratios for 21-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, and 11β-hydroxysteroid dehydrogenase-2. They included a large number of samples from healthy infants (n = 324) aged 3 days to 6 months, overcoming the limitations of previous studies that had small numbers by sex and age. Moreover, four patients with molecularly proven 21-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, and aldosterone synthase deficiencies were included to test the steroid panel's diagnostic efficacy. Due to the larger sample size, changes in steroids concentrations over time could also be observed. Most steroids showed age-related variations that could be attributed to the changes in adrenal morphology and steroidogenic enzyme expression.

In summary, the authors provide a detailed set of normative reference values for steroidogenesis during the first 6 months of life. This and similar future datasets may facilitate rapid testing of infants in neonatal screening for steroidogenic disorders.

8.6. Characteristics of growth in children with classic Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency during adrenarche and beyond

Troger T, Sommer G, Lang-Muritano M, Konrad D, Kuhlmann B, Zumsteg U, Flück CE
J Clin Endocrinol Metab. 2022; 107(2): e487-e499.
PMID: 34599587
https://pubmed.ncbi.nlm.nih.gov/34599587/

Brief Summary: This study describes the growth of adequately treated children with classical Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency during adrenarche and beyond. Patients with and without significant bone age advancement, and thus differing height prediction during adrenarche, showed similar (predicted) final height when reassessed during puberty.

Patients with classical CAH often do not achieve their full growth potential due to the difficulties in balancing the effects of hyperandrogenism and hypercortisolism during childhood and adolescence (**1**). Whether the increased height velocity during adrenarche has any impact on final height in this patient group is still not clear (**2, 3**).

This study describes the bone age, growth velocity/height and predicted final height during puberty and adrenarche in 41 children (26 girls) with classical CAH. Longitudinal data were grouped in three time periods: A) Before adrenarche, aged < 6 yrs; B) Adrenarche, > = 6 yrs to pubertal onset; C) Puberty, after the onset of puberty. Patients were categorized into two groups during adrenarche: those with accelerating bone age (49%; BA-CA at least 0.1 year higher at age 6-9 yrs than < 6 yrs of age) or non-accelerating bone age (all others). At the time of the study, 37/41 patients had developed pubarche (P2) at a mean age of 10.5 yrs in girls (B2 at 10.3 yrs) and 11.7 yrs in boys (G2 at 12.1 yrs). The hydrocortisone (HC) dose was between 14.1 to 16.2 mg/m^2/day, while 40/41 patients also received fludrocortisone.

Patients were shorter than peers (−0.4 SDS ± 0.8 SD) and than their parental target (corrected final height −0.6 SDS ± 1.0 SD). Children with accelerated BA-CA were taller during adrenarche than those without accelerating BA-CA (mean height SDS 0.5 vs. -0.3 SDS). However, these differences disappeared during puberty. Growth velocity was higher before adrenarche in children with accelerating BA-CA and decreased thereafter. Estimated final height was lower in those with accelerating BA-CA but this difference disappeared at puberty. In the end, final adult height was similarly reduced in both accelerating BA-CA and non-accelerating BA-CA groups (-0.4 SDS vs. -0.3 SDS).

In summary, patients with classical CAH achieve a final height that is close to normal. Although a growth acceleration and bone age progression can be observed in almost 50% of children during the years of

adrenarche, this does not result in a more compromised (predicted) final height compared with patients without such bone age progression. These findings indicate that bone age alone should not be used during adrenarche as a clinical marker for metabolic control in patients with classic CAH.

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8.7. Low adrenomedullary function predicts acute illness in infants with classical Congenital Adrenal Hyperplasia

Weber J, Tanawattanacharoen VK, Seagroves A, Liang MC, Koppin CM, Ross HM, Bachega TASS, Geffner ME, Serrano-Gonzalez M, Bhullar G, Kim MS

J Clin Endocrinol Metab. 2022; 107(1): e264–e271.

PMID: 34397083

https://pubmed.ncbi.nlm.nih.gov/34397083/

Brief Summary: This prospective cohort study determined plasma epinephrine concentrations in infants with classic CAH, and showed that the lower epinephrine reseve is associated with increased risk of illness.

Children and adolescents with classical Congenital Adrenal Hyperplasia (CAH) have impaired adrenomedullary function with decreased epinephrine concentrations noted in newborns and young infants. This study measured plasma epinephrine concentrations in infants with classic CAH and related these to morbidity during the first year of life. The authors studied prospectively 36 infants with classical CAH due to 21-hydroxylase deficiency and 27 age-matched unaffected controls (with congenital hypothyroidism). Main outcomes included plasma epinephrine concentrations (n = 27), CYP21A2 genotype (n = 15), and incidence of acute illnesses from birth to age 1 year (n = 28).

Higher epinephrine concentrations in infants with CAH were negatively correlated with 17-hydroxyprogesterone at diagnosis ($R = -0.51$, $p = 0.007$) and independently predicted less illness in the first year of life ($\beta = -0.018$, $R = -0.45$, $p = 0.02$). Infants with salt-wasting CAH had lower epinephrine concentrations as newborns than simple-virilizing infants. Patients with CAH had lower epinephrine as newborns than controls, and showed decreases in epinephrine from birth to age 1 year. Null genotype was associated with lower newborn epinephrine concentrations and more incidences of illness in the first year of life, compared to less severe mutation categories.

In summary, these findings show that lower epinephrine concentrations are associated with increased risk of illness in infants with classic CAH. Therefore, determining epinephrine concentrations may help predict acute illness in the first year of life.

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Clinical Trials - New Treatments

8.8. Crinecerfont lowers elevated hormone markers in adults with 21-hydroxylase deficiency congenital adrenal hyperplasia

Auchus AR, Sarafoglou K, Fechner PY, Vogiatzi MG, Imel EA, Davis SM, Giri N, Sturgeon J, Roberts E, Chan JL, Farber RH

J Clin Endocrinol Metab. 2022; 107(3): 801-812.

PMID: 34653252

https://pubmed.ncbi.nlm.nih.gov/34653252/

Brief Summary: This clinical trial evaluated the safety and efficacy of *crinecerfont*, a CRF1R antagonist, in suppressing adrenal androgen secretion in adult patients with classic congenital adrenal hyperplasia (CAH) during a treatment period of 14 days.

Classical CAH due to 21-hydroxylase deficiency (21OHD) is characterized by impaired cortisol synthesis and excess adrenal androgen secretion. Sufficient suppression of adrenal andogen production may be difficult (**1**). Novel therapeutic approaches with corticotropin-releasing factor type 1 receptor (CRF1R) antagonists such as crinecerfont could overcome this difficult. CRF1R antagonists decrease ACTH concentrations, thereby suppressing adrenal androgen excess and avoiding the need for supraphysiologic doses of glucocorticoids (**2, 3**).

In this phase-2 open label study, the CRF1R antagonist crinecerfont was administered orally to 18 adults with classical CAH (age range 18-50 yrs; mean age 31 yrs) for 14 days. Inclusion criteria were: BMI 18-45 kg/m^2; Serum 17OHP in the morning prior to GC medication $>=$ 30.3 nmol/L; Serum cortisol $<$ 138 nmol/L; Plasma ACTH $>=$ 4.4 pmol/L. All patients also continued their normal daily GC regimen. Outcome measures included ACTH, 17OHP, A4 and testosterone at baseline and after 14 days of treatment with either 50 or 100 mg of crinecerfont at bedtime; 100 mg in the evening; and 100 mg twice daily.

The highest crinecerfont dose reduced ACTH, A4 and 17OHP concentrations by ~60%. In females, testosterone concentrations decreased by 50% in most participants (8/11), while males there showed a substantial decrease in A4/testosterone ratios. Dose-response effects were seen, with the largest effect seen on A4 concentrations. No severe adverse effects were reported.

In summary, crinecerfont treatment for 14 days decreased ACTH concentrations and afforded clinically meaningful reductions of elevated 17OHP, androstenedione, testosterone (women), or androstenedione/testosterone ratio (men) in adults with 21OHD. Longer-term studies are required to evaluate the effects of crinecerfont on clinical end points of disordered steroidogenesis and glucocorticoid exposure in patients with 21OHD. Studies in children and adolescents with classic CAH are on-going.

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8.9. Tildacerfont in adults with classic Congenital Adrenal Hyperplasia: Results from two phase 2 studies

Sarafoglou K, Barnes CN, Huang M, Imel EA, Madu IJ, Merke DP, Moriarty D, Nakhle S, Newfield RS, Vogiatzi MG, Auchus RJ
J Clin Endocrinol Metab. 2021; 106(11): e4666-e4679.
PMID: 34146101
https://pubmed.ncbi.nlm.nih.gov/34146101/

Brief Summary: These clinical trials evaluated the safety and efficacy of *tildacerfront*, a CRF1R antagonist, in suppressing adrenal androgen secretion in adult patients with classical Congenital adrenal hyperplasia (CAH) during a treatment period of 12 weeks.

CAH due to 21-hydroxylase deficiency (21OHD) is typically treated with lifelong supraphysiologic doses of glucocorticoids (GCs) (**1**). Tildacerfont, a corticotropin-releasing factor type-1 receptor (CRF1R) antagonist, may reduce excess adrenal androgen production, thus allowing the use of lower GC doses (**2-4**). In these two Phase 2 open-label studies, the authors evaluated the efficacy and safety of tildacerfont. In study 1, doses of 200 mg to 1000 mg once daily (escalating doses) or 100 mg or 200 mg twice daily were administered orally for 14 days to adults with CAH. In study 2, the participants received 400 mg once daily for 12 weeks. In total, 36 participants were enrolled (age range 19-67 yrs; mean age 45 yrs) provided their morning concentrations of serum 17OHP concentrations prior to GC medication were $>=$ 24 nmol/L. No severe adverse events were

noted. Eleven participants were on dexamethasone and were excluded from the efficacy analysis since the concentrations of dexamethasone in blood were doubled during the tildacerfont treatment period. In the group of the remaining 25 participants, 11/16 in study 1 and 5/8 in study 2 were considered to have poor disease control at baseline. After receiving tildacerfont for 14 days, the morning concentrations of ACTH, 17OHP and A4 were reduced in the poor disease control group. No dose-response relationship was observed across the evaluated dose range. For participants with good disease control (ACTH and A4 below the upper normal reference range) there were no additive effects of tildacerfont on ACTH or A4 concentrations, and only a modest decrease in 17OHP concentrations was noted. In the 12-week study group (study 2, 400 mg/day) a similar result was observed for the biomarker reduction in the poor disease-control group, while the good disease-control group showed no benefit of tildacerfont. Whether tildacerfont has the power to reduce GC doses further in the good disease-control group remains to be tested.

In conclusion, these open label studies demonstrate proof of concept the achievement of receptor engagement (reductions in ACTH) and suppression of adrenal steroids (17-OHP and A4) in 21OHD CAH. In Study 1, the mean levels of ACTH, 17-OHP, and A4 were reduced relative to baseline across all doses tested. Study 2 showed that continued reductions could be achieved across each of the key biomarkers with longer term therapy, including normalization of ACTH and A4 levels in patients with poor baseline control. Tildacerfont was generally well-tolerated and safe. The findings from these studies support the ongoing late-stage studies of tildacerfont in participants with 21OHD.

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2. Chen C. Recent advances in small molecule antagonists of the corticotropin-releasing factor type-1 receptor-focus on pharmacology and pharmacokinetics. *Curr Med Chem*. 2006; 13(11): 1261–1282.
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New Hope

8.10. The brain penetrant PPARγ agonist leriglitazone restores multiple altered pathways in models of X-linked adrenoleukodystrophy

Rodríguez-Pascau L, Vilalta A, Cerrada M, Traver E, Forss-Petter S, Weinhofer I, Bauer J, Kemp S, Pina G, Pascual S, Meya U, Musolino PL, Berger J, Martinell M, Pizcueta P

Sci Transl Med. 2021; 13(596): eabc0555.

PMID: 34078742

https://pubmed.ncbi.nlm.nih.gov/34078742/

Brief Summary: These *in vitro* and *in vivo* studies show that the brain penetrant PPARγ agonist *leriglitazone* restores multiple biological pathways relevant for neuroinflammatory and neurodegenerative diseases, and particularly for X-linked adreno-leukodystrophy (X-ALD).

Peroxisome proliferator-activated receptor gamma (PPARγ) agonists act on multiple pathways through gene activation or repression, and have the capacity to induce neuroprotective and restorative effects in several preclinical models of neurodegenerative diseases, such as Amyotrophic lateral sclerosis, Parkinson's disease, Friedreich's ataxia, Alzheimer's disease and adrenomyeloneuropathy (AMN) (**1**). Leriglitazone is a PPARγ agonist developed by Minoryx Therapeutics for the treatment of X-ALD and other neurodegenerative diseases due to its blood-brain-barrier (BBB) penetration, good bioavailability and safety profile (**2**). Leriglitazone, also known as MIN-102, is the hydrochloride salt of the active metabolite M4 (M-IV) of pioglitazone (Actos, Takeda).

These authors performed several experiments in rodent primary neurons, astrocytes, endothelial cells, oligodendrocytes and microglia. Leriglitazone efficacy in treating X-ALD was further validated *in vivo* in murine AMN models and in mice with Experimental Autoimmune Encephalomyelitis (EAE), a surrogate

model for the neuroinflammatory component of cerebral adrenoleukodystrophy (cALD). To better understand the mode of action of leriglitazone in potentially preventing early stages of the development of cALD, the authors used *in vitro* models and showed a 50% increase in the brain/plasma exposure ratio compared to pioglitazone in mice, while the unbound fraction of leriglitazone in brain also increased to 9.1 and 13.6% compared to 1.6 and 1.2% of pioglitazone. The authors further showed protective effects of leriglitazone from VLCFA-induced toxicity in an *in vitro* model simulating X-ALD, in mouse models of AMN. In models of blood-brain barrier mimicking X-ALD conditions, by challenging the brain endothelium/astrocytes with an inflammatory stimulus, or in monocytes/macrophages derived from patients with X-ALD, they showed anti-inflammatory effects of leriglitazone. Furthermore, the protective effects of leriglitazone against demyelination and/or enhancement of remyelination *in vitro* and *in vivo*. Finally, a Phase I pharmacodynamic/pharmacokinetic study in healthy volunteers was completed to confirm inflammatory biomarker changes and target engagement in plasma and CSF in humans at concentrations corresponding to preclinical efficacious doses.

This is an important study, highlighting the potential of leriglitazone, particularly as no effective pharmacological treatments are currently available for X-ALD. Hematopoietic Stem Cell Transplantation (HSCT) or experimental gene therapy can be used to arrest the cerebral form of X-ALD by counteracting activated microglia with differentiated new macrophages/microglia from hematopoietic stem cells. However, delay in diagnosis, lack of appropriate donors and adverse events associated with transplantation make HSCT only available and effective to a minority of patients with cALD. In addition, HSCT does not avoid the progression to AMN in a later stage.

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New Concerns

8.11. Cortisol and development of depression in adolescence and young adulthood - a systematic review and meta-analysis

Zajkowska Z, Gullett N, Walsh A, Zonca V, Pedersen GA, Souza L, Kieling C, Fisher HL, Kohrt BA, Mondelli V

Psychoneuroendocrinology. 2022; 136: 105625.

PMID: 34920399

https://pubmed.ncbi.nlm.nih.gov/34920399/

Brief Summary: This is a systematic review and meta-analysis examined the relationship between cortisol and major depressive disorder in global youth (10–24 years old).

Impaired regulation of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the development of major depressive disorder (MDD) in adulthood, however, limited information exists on the role of the HPA axis in depression in adolescence and young adulthood (**1, 2**). These authors conducted a systematic review and meta-analysis of research investigating the relationship between cortisol and major depressive disorder (MDD) in adolescence and young adulthood. An association between the dysregulation of the HPA axis and the pathophysiology of MDD has been suggested in the past, especially in adults.

The authors found that elevated morning, but not evening, cortisol concentration was prospectively associated with later MDD development in adolescence and young adulthood. Qualitative synthesis of the three studies examining nocturnal cortisol showed that higher nocturnal cortisol was both longitudinally and cross-sectionally associated with MDD in adolescence. However, morning cortisol concentrations did not significantly differ between healthy controls and subjects with MDD in cross-sectional studies. Afternoon cortisol and cortisol stress response also did not differ between adolescents with MDD and healthy controls.

These data suggest that elevated morning cortisol concentrations precede subsequent MDD onset in adolescence, regardless of being a first or recurrent episode of depression. This may suggest that elevated cortisol might be a

predictor rather than a consequence of depression. Furthermore, cumulative exposure to stress which initially results in elevated cortisol, with time, leads to blunted cortisol response which overlaps with MDD onset.

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New Paradigms

8.12. Glucocorticoid-induced fingerprints on visceral adipose tissue transcriptome and epigenome

García-Eguren G, González-Ramírez M, Vizán P, Giró O, Vega-Beyhart A, Boswell L, Mora M, Halperin I, Carmona F, Gracia M, Casals G, Squarcia M, Enseñat J, Vidal O, Di Croce L, Hanzu FA

J Clin Endocrinol Metab. 2022; 107(1): 150-166.
PMID: 34487152
https://pubmed.ncbi.nlm.nih.gov/34487152/

Brief Summary: This translational study determined the persistent visceral adipose tissue (VAT) transcriptomic alterations and epigenetic fingerprints induced by chronic hypercortisolism in patients with Cushing's syndrome (CS) and in a reversible CS mouse model.

Excessive and prolonged glucocorticoid (GC) exposure may result in diverse and long-term adverse outcomes. Patients with Cushing's Syndrome (CS, endogenous GC excess) have persistently increased cardiometabolic risk, with abdominal fat accumulation and systemic low-grade inflammation. GCs play an important role in adipose tissue metabolism, however, the effects on transcriptomic and epigenomic changes have not been studied extensively in this patient group.

These authors described these effects using a translational approach, including both visceral adipose tissue (VAT) biopsies from patients with active CS and matched controls, and a mouse model of reversible hypercortisolism. The authors performed RNA sequencing and chromatin immunoprecipitation sequencing on the histone modifications H3K4me3, H3K27ac, and H3K27me3 to identify persistent transcriptional and epigenetic signatures in VAT produced during active CS and after remission. They found similar transcriptomic changes in both humans and mice with differentially expressed genes being related to inflammation and regulation of transcription. More importantly, GC exposure altered the expression of genes associated with controlling circadian rhythm or disrupting it. Furthermore, the results regarding histone modifications were different from controls in both mice and humans, showing opposite effects, with mice showing increased signals from all three modifications and humans showing the opposite effect. Observed changes in both H3K4me3 and H3K27ac, histone modifications that are markers for gene-activation, correlated with the differences observed in gene expression in active CS and after remission, indicating that chronic GC exposure alters the epigenetic profile of VAT that persists through time with important implications for gene expression and downstream biological functions.

In summary, the article explores and describes the effects of GCs on VAT both during and after hypercortisolism. Hypercortisolism has numerous and various adverse effects depending on time and the magnitude of the exposure. Understanding the biological effects of excess GC exposure in different tissues and the underlying mechanisms of these effects may be helpful in the future clinical management.

Reviews

8.13. Diagnosis and treatment of primary aldosteronism

Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA

Lancet Diabetes Endocrinol. 2021; 9(12):876-892.
PMID: 34798068
https://pubmed.ncbi.nlm.nih.gov/34798068/

Brief Summary: This review summarizes the current knowledge on the epidemiology, genetic background, pathophysiology, clinical presentation, diagnosis and treatment of primary aldosteronism.

This review article on primary aldosteronism (PA) describes its clinical characteristics and the diagnostic process of screening, confirmation, and subtyping. Furthermore, the authors highlight the current standards of treatment and discuss controversies and areas of uncertainty.

The importance of awareness of PA owes to the significant comorbidities associated with aldosterone excess on the heart, vessels, brain, and kidney, which are partly independent of elevated blood pressure. These arguments strongly support the early and systematic detection of PA, in order to implement efficient surgical or medical treatment, to prevent or reverse the comorbidities in this specific group of patients with secondary hypertension. Adult patients with moderate to severe hypertension or those with hypertension and spontaneous or diuretic-induced hypokalaemia, adrenal incidentaloma, atrial fibrillation in the absence of structural heart disease, or a family history of early onset hypertension or stroke at a young age (<40 years), as well as all first-degree relatives with hypertension of patients with primary aldosteronism, are candidates for screening. It is important to note that screening prior to commencing antihypertensive therapy has the benefit of avoiding the potentially confounding effects of these agents on renin and aldosterone concentrations, and allows the earlier initiation of specific treatment.

In summary, PA is characterized by the paradox of being a common cause and augmenter of hypertension, with a high incidence, that is underdiagnosed in most health-care systems. Affected patients can potentially develop resistant hypertension associated with a low quality of life and adverse cardiovascular and cerebrovascular outcomes. The authors argue that PA should be regarded as a distinct cardiovascular risk factor, alongside other classic risk factors (e.g., diabetes mellitus, hypercholesterolaemia, and smoking) that aggravate and potentiate adverse outcomes of hypertension. Thus, instead of screening selected at-risk populations, general screening of people with hypertension should be the future strategy to reduce the disease-burden in a cost-effective way. These concepts are of particular importance to Pediatric Endocrinologists because there is higher incidence of PA in hypertensive patients who are routinely screened, and idiopathic hypertension is relatively rare.

8.14. Should Dehydroepiandrosterone be administered to women?

Wierman ME, Kiseljak-Vassiliades K
J Clin Endocrinol Metab. 2022; 107(6): 1679-1685.
PMID: 35254428
https://pubmed.ncbi.nlm.nih.gov/35254428/

Brief Summary: This review summarizes the evidence on the potential benefits and risks of androgen prohormones, such as dehydroepiandrosterone (DHEA), in normal women and those with DHEA-deficient states.

Physiologically, the concentrations of DHEA and DHEAS increase during adrenarche and throughout puberty, and peak in the late 20s to 30s before declining with age, independent of menopausal status. This narrative review discusses the issues surrounding Dehydroepiandrosterone (DHEA) supplementation, given that DHEA is currently available over the counter or via the internet in many countries, as a supplement outside of the remit of medicines regulatory bodies, such as FDA or EMA. However, quality control of DHEA is inconsistent, while data are lacking on the physiologic dose of this hormone and the benefit of DHEA in women with adrenal insufficiency at any age. In premenopausal women with low DHEAS concentrations, one could argue that DHEA therapy might have potential benefits on wellbeing, understanding that the physiologic dose of DHEA in women is somewhere around 25 and not 50 mg/day. In postmenopausal women, one must consider the additional impact of conversion to testosterone and estradiol to her breast and bone health and the cardiac risks, and discuss the pros and cons of a short-term trial. Long-term supplementation in DHEA deficient states is not well-established, while there is much discussion on DHEA supplementation in non-deficient states. Among the non-deficient states, DHEA supplementation has no clear benefit on anti-aging effects, physical and psychological wellbeing, libido, cognition and perimenopausal symptoms. Other conditions where DHEA

supplementation is discussed include anorexia nervosa (where small studies show potential benefits), mood disorders (no suggested benefit), bone health (effects on bone in women are less than estrogen or other FDA-approved osteoporosis medications, and no data available on fracture risk). The studies on the potential metabolic effects, genitourinary symptoms, or infertility have not provided sufficient data to suggest that it should be used consistently.

8.15. The genetics of autoimmune Addison disease: past, present and future

Ellen C, Røyrvik EC, Husebye ES
Nat Rev Endocrinol. 2022; 18(7): 399-412.
PMID: 35411072
https://pubmed.ncbi.nlm.nih.gov/35411072/

Brief Summary: This review summarizes the current knowledge and understanding of the genetics of autoimmune Addison disease and its position in the wider field of autoimmune disorders.

Autoimmune Addison's disease (AAD) is caused by the destruction of the adrenal cortex causing deficiencies of certain adrenal steroids. Patients with AAD require life-long replacement therapy with corticosteroids. It has long been recognized that AAD has an important genetic component and attempts at identifying genetic variants that may explain individual predisposition have been performed over the last two decades. This review summarizes the current knowledge and recent advances regarding the genetic etiology of autoimmune Addison's disease. The authors highlight that previous studies on this topic had problems with reproducibility, an issue that might be solved with more unbiased genome-wide association studies. Furthermore, the review thoroughly describes known AAD risk loci, both from candidate gene and genome-wide association studies. Plausible risk loci indicate that AAD is primarily a T cell-mediated disease, with affected individuals predisposed to it because they carry common variants that individually are only mildly deleterious on autoimmunity. Many AAD risk loci overlap with those published for other autoimmune diseases, such as type 1 diabetes, indicating that the diseases have similar pathogenic mechanisms.

In summary, the review provides a good overview regarding the genetic etiology of AAD. The authors describe the associations between plausible risk loci and disease mechanisms, and provide useful suggestions about future studies to determine the etiology of AAD.

8.16. Management challenges and therapeutic advances in congenital adrenal hyperplasia

Mallappa A, Merke DP
Nat Rev Endocrinol. 2022; 18(6): 337-352.
PMID: 35411073
https://pubmed.ncbi.nlm.nih.gov/35411073/

Brief Summary: This review summarizes the current knowledge and understanding of the therapeutic challenges and the novel advances in the management of classical congenital adrenal hyperplasia (CAH).

This review describes the present and novel therapeutic options for 21OHD CAH. Several novel approaches to address the lack of normal cortisol circadian rhythm and hyperandrogenism in CAH are plausible future targets for intervention. Among those are: i) Modified release hydrocortisone (Chronocort); ii) Adrenal steroidogenesis inhibitors (abiraterone, nevanimibe); iii) HPA axis suppressors (CRF1R antagonists, ACTH antagonist - monoclonal antibody, mC2R antagonist); iii) Cell-based therapies - human induced steroidogenic cells and transplantation of bioartificial adrenal cortex; and iv) Gene-based therapies - Adeno-associated virus-based gene therapy, BBP-631 (currently ongoing phase I–II trials).

Chronocort was investigated in a multicenter phase III, randomized, parallel arm study in 122 adults with classic CAH, conducted over 24 weeks. The trial missed its primary end point as Chronocort was not superior to

standard glucocorticoid therapy based on the 24-hour profile of serum 17OHP concentrations. However, compared with standard glucocorticoid therapy, Chronocort improved 17OHP and androstenedione concentrations in the morning and early afternoon, with a decrease in daily hormonal fluctuations. At 18 months extension, further benefits included: improved menstruation ($n = 4$) and patient or partner pregnancy ($n = 5$). Chronocort (brand name Efmody) received marketing approval in 2021 in the UK and Europe for patients with CAH aged 12 years and older.

Abiraterone is a potent CYP17A1 inhibitor that lowers testosterone production and is used to treat prostate cancer. Abiraterone was studied as a phase I, non-randomized, open-label, multiple-dose (6 days), sequential dose-escalation (100–250 mg once-daily) trial in six women (aged 19–46 years) with classical CAH. Abiraterone decreased serum concentrations of testosterone, and 11-oxygenated androgens and urinary androgen metabolites. A phase I–II study in prepubertal children is underway.

Potential strategies to address the drivers of excess androgen synthesis in CAH include molecules that antagonize the action of CRF1, a monoclonal antibody to ACTH and a selective MC2R antagonist. The CRF1R antagonists, crinecerfont and tildacerfont, are described in papers 8.8 and 8.9 of this chapter.

The first feasibility study of gene therapy for CAH used a replication-deficient adenovirus containing human *CYP21A2*in 21OHD knockout mice. An intra-adrenal injection of the vector restored adrenocortical function for up to 40 days. A subsequent study of an intravenous injection of an AAV-*CYP21A2*vector in CAH mice restored steroidogenesis for > 15 weeks. Intramuscular injection of an AAV vector with mouse *Cyp21a1*in CAH mice resulted in enzyme expression for > 6 months. BBP-631 (Adrenas Therapeutics, USA) is an AAV5 vector encoding human *CYP21A2*. Preclinical durability studies of a single intravenous injection in CAH mice and cynomolgus monkeys demonstrated adrenal tropism of the vector and dose-dependentRNA expression for 12 weeks and durable expression of vector genome copies up to 24 weeks. A phase I–II, open-label, dose-escalation trial investigating the safety and efficacy of gene therapy for adults with classic CAH is under way.

In summary, multiple hormonal imbalances complicate the management of CAH. Future research in CAH aims to tailor therapy to maximize clinical benefits and minimize long-term adverse outcomes. The multitude of new advances in glucocorticoid replacement therapy, glucocorticoid-sparing adjuvant therapies, and cell-based and gene-based therapies promises an improved outcome in patients with CAH.

Food for Thought

8.17. The mediating pathways between parental separation in childhood and offspring hypertension at midlife

Stannard S, Berrington A, Alwan NA
Sci Rep. 2022; 12(1): 7062.
PMID: 35488035
https://pubmed.ncbi.nlm.nih.gov/35488035/

Brief Summary: This study provides evidence on the relationship between parental separation and hypertension at midlife and suggests interventions to enhance the psychological and cognitive development of girls who have experienced parental separation to help reduce multiple adverse health outcomes, including hypertension in adulthood.

Life events in early life may shape health trajectories. Parental separation and the subsequent adjustment reflect disruption that can continue for many years. Previous studies focused mostly on outcomes in early adulthood. Few studies extended the time frame to midlife. Studies that investigated outcomes in older adulthood suggest that offspring who experience parental separation may be at an increased risk of cardiovascular disease, obesity and stroke, and an increase in mortality risk.

This study examined whether parental separation in childhood is associated with hypertension at age 46, whether this differs by gender, and how any such association is mediated through family socioeconomic status (SES)

during childhood, child behavior and cognitive development, and childhood physical health. It used prospective longitudinal data and formal mediation analysis to quantify previously unexplored mediating pathways in childhood, reflecting on how early life mediators might lead into adult mediators of hypertension, as a key early life event known to have significant implications for children's outcomes in education, mental wellbeing and physical health. Furthermore, this analysis suggests a significant gender difference in the association between parental separation and offspring hypertension. While no association was found for men, for women parental separation was associated with offspring hypertension (in unadjusted models). Girls who experience parental separation before age 10 years may suffer a decline in economic and social resources, and poorer motor coordination and behavioral development, which predicts later health outcomes. These childhood mediators appear to partially mediate the association between parental separation and hypertension at age 46. Although, these childhood mediators partly operate through adult mediators, policy interventions should still consider these childhood antecedents in prevention efforts. Parental separation is stressful process and exposure to chronic stressors during early developmental years can lead to both long-term elevated blood pressure and long-lasting neurobiological effects, including a decline in cognitive development and an increase in behavioral problems.

In summary, this study provides evidence on the relationship between parental separation and hypertension at midlife. The findings suggest interventions to enhance the psychological and cognitive development of girls who have experienced parental separation to help reduce multiple adverse health outcomes, possibly including hypertension in adulthood.

8.18. Associations between testosterone, estradiol, and androgen receptor genotype with amygdala subregions in adolescents

Campbell CE, Mezher AF, Tyszka JM, Nagel BJ, Eckel SP, Herting MM
Psychoneuroendocrinology. 2022; 137: 105604.
PMID: 34971856
https://pubmed.ncbi.nlm.nih.gov/34971856/

Brief Summary: This study evaluated the associations between the amygdala total and sub-region volumes in relation to sex hormones – estradiol and free testosterone – as a function of age and genetic differences in androgen receptor sensitivity in 297 adolescents. It provides new knowledge regarding the influence of sex hormones on amygdala sub-regions.

The amygdala is well-studied in many aspects. However, previous studies on the effect of sex steroid hormones during development and amygdala sub-region volumes show mixed results. These authors investigated the effects of sex hormones (free testosterone and estradiol) on amygdala volumes using magnetic resonance imaging (MRI) in 297 adolescents aged 10-17 years. Androgen receptor (AR) CAG repeat number was assessed as a marker of transcriptional activity and androgen sensitivity.

They did not find any overall relationship between amygdala volumes and sex hormones or AR sensitivity. However, sex-specific differences were observed. Specifically, amygdala volumes in females were more influenced by AR genetic variation, where lower AR sensitivity was associated with larger basolateral complex volumes. In males, the interactions of testosterone, age, and AR polymorphism were more relevant for the amygdala volumes in the basolateral complex and cortico-medial subnuclei. The authors speculate that this may be partially explained by differing expression levels of AR, aromatase expression, or possibly both, between amygdala subregions. This is the first study to investigate whether genetic variation in the AR may independently or jointly interact with hormone concentrations and/or age influencing the amygdala and its subregions volumes in adolescents. While the study provides new knowledge regarding the influence of sex hormones on amygdala subregions, the effects observed are small and need to be confirmed.

9. Oncology and Chronic Disease

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Introduction

As in previous years, most of the data published this year focuses on the topic of fertility preservation in childhood cancer survivors.

New studies have shown that the reproductive capacity of these patients is now significantly increased compared to what was reported in previous decades. This improvement is clearly related to improved fertility preservation and assisted reproduction techniques. Another relevant factor is the progressive personalization of antineoplastic therapy protocols, based on the characterization of the patient's genetic profile and the gene expression pattern of the neoplasm itself. This has allowed not only greater efficacy and tolerability of antineoplastic therapy protocols, but also a reduction in chronic complications of cancer treatment, particularly gonadotoxicity and damage to other endocrine glands. In this regard, preliminary data are emerging that would correlate specific polymorphisms in genes encoding enzymes that regulate the metabolism of antineoplastic drugs with the magnitude of the risk of endocrine complications, particularly gonadal damage.

Cancer Treatment and Growth Hormone Therapy: A Consensus

9.1. Safety of growth hormone replacement in survivors of cancer and intracranial and pituitary tumours: a consensus statement

Boguszewski MCS, Boguszewski CL, Chemaitilly W, Cohen LE, Gebauer J, Higham C, Hoffman AR, Polak M, Yuen KCJ, Alos N, Antal Z, Bidlingmaier M, Biller BMK, Brabant G, Choong CSY, Cianfarani S, Clayton PE, Coutant R, Cardoso-Demartini AA, Fernandez A, Grimberg A, Guðmundsson K, Guevara-Aguirre J, Ho KKY, Horikawa R, Isidori AM, Jørgensen JOL, Kamenicky P, Karavitaki N, Kopchick JJ, Lodish M, Luo X, McCormack AI, Meacham L, Melmed S, Mostoufi Moab S, Müller HL, Neggers SJCMM, Aguiar Oliveira MH, Ozono K, Pennisi PA, Popovic V, Radovick S, Savendahl L, Touraine P, van Santen HM, Johannsson G
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Eur J Endocrinol. 2022; 186: P35-P52.
PMID: 35319491.

Brief Summary: An international panel of experts produced this consensus statement after critical review of the existing evidence on the safety of GH replacement in cancer and intracranial tumour survivors and in patients with increased cancer risk.

Current evidence does not support an association between GH replacement and primary tumour or cancer recurrence, with more robust data for adults with benign pituitary adenoma and craniopharyngioma. GH replacement should be discontinued when disease relapse or clinically significant tumour progression is confirmed. Resumption of GH replacement could be considered after discussion between clinicians, patient and caregivers one year after further remission. A shorter waiting time may be acceptable for non-malignant tumours and craniopharyngioma. The risk of secondary neoplasia, mainly meningiomas, is less related to GH replacement than to cancer treatments (e.g. radiotherapy), and the suggested timing of GH discontinuation is similar to cancer relapse. Current evidence does not support an association between GH treatment and increased mortality from cancer among childhood cancer survivors with GHD. IGF-I has limited reliability as a marker of GHD in cancer survivors. Furthermore, dynamic testing with GHRH should be avoided in patients who received cranial irradiation, due to possible false negative results, despite the complex disruption of the hypothalamic-

pituitary axis. The timing of initiation of GH therapy following completion of cancer treatment should be individualized, as a decision shared by treating physicians, patient, and caregivers. This period may be as early as 3 months in children with radiologically-proven stable craniopharyngiomas who have significant growth failure and metabolic disturbances, and at least 1 year for other types of tumours. A low starting GH dose is suggested, with IGF-1 measurements every 3 months during dose titration and annually thereafter. Increased intracranial pressure, slipped capital femoral epiphysis, and worsening of scoliosis are GH-related side effects that are more common in cancer survivors. In children with cancer predisposition syndromes (including RASopathies such as Noonan syndrome), GH treatment is generally contraindicated, but it may be considered cautiously in cases with proven GHD.

The cautious statements of this extensive and updated consensus represent a useful tool to support physicians, patients and their families in decision-making on GH replacement, in particular in the areas of uncertainly due to lack of data. The position of the consensus concerning GH therapy for patients with RASopathies is noteworthy. While GH treatment has recently been approved in several countries for treatment of children with Noonan syndrome and short stature, regardless of the presence or absence of GHD (1), these experts suggest cautiously considering GH treatment in patients with RASopathies, only in the presence of a proven GHD.

Reference

1. Jorge AAL, et al. Outcomes in growth hormone-treated Noonan syndrome children: impact of PTPN11 mutation status. *Endocr Connect*. 2022. PMID: 35245205.

Thyroid Cancer in Childhood Cancer Survivors

9.2. Surgical outcomes in survivors of childhood cancer undergoing thyroidectomy: A single-institution experience

Van Remortel BJ, Chehab L, Bauer AJ, Isaza A, Yimei L, Baumgarten HD, Franco AT, Laetsch TW, Kazahaya K, Adzick NS, Mostoufi-Moab S

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Pediatr Blood Cancer. 2022; 69: e29674.

PMID: 35338690.

Brief Summary: Childhood cancer survivors (CCS), particularly those exposed to local irradiation, are at increased risk for thyroid disease and thyroid surgery. This retrospective, monocentric study compared rates of surgical complications among 42 CCS undergoing thyroid surgery compared to 596 non-CCS controls (sporadic/familial thyroid cancer, Graves' disease, other benign thyroid conditions).

In the CCS group, median age at surgery was higher (17 vs 15 years) and the proportion of females was lower (55% vs 82%). 85% of patients underwent total thyroidectomy (vs 76% of sporadic/familial thyroid cancer group, 32% of benign conditions group). Only 43% of CCS underwent lymph-node dissection vs 74% of patients with sporadic/familial thyroid cancer. Median operative times in CCS group were shorter compared to patients with sporadic/familial thyroid cancer (92 min vs 130 min for total thyroidectomy) and similar to patients with benign conditions. There was no difference in the proportions of low-intermediate- or high-risk disease, compared with sporadic/familial differentiated thyroid cancer (DTC). There was no differences in the incidence of postoperative complications after total or partial thyroidectomy between CCS and controls. CCS showed an increased incidence of transient but not permanent hypoparathyroidism (71% vs 25%).

Contrary to what might be expected, CCS did not show a higher risk of long-term complications from thyroid surgery, when treated by high-volume surgeons within a multidisciplinary team. The higher rates of transient hypoparathyroidism in CCS, according the Authors, were likely secondary to a greater proportion of total thyroidectomy and parathyroid auto-transplantation than in patients with benign conditions. The shorter operative times may be related to an earlier detection of thyroid nodules in CCS, due to the regular ultrasound screening performed in these patients.

The large sample size of CCS patients and controls represents the main strength of this study. Possible limitations are related to data source from a single-center experience, and to the retrospective design of the study.

9.3. Characterization and risk factors of hyperglycaemia during treatment of childhood hematologic malignancies

Welsch S, Sawadogo K, Brichard B, de Ville de Goyet M, Van Damme A, Boulanger C, Lysy P
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Diabet Med. 2022; 39: e14720.
PMID: 34652870.

Brief Summary: Childhood cancer survivors are at increased risk of hyperglycaemia and diabetes due to cancer treatments as asparaginase, steroids and total body irradiation. This single center, retrospective study analyzed the prevalence of hyperglycaemia in 267 patients treated between 2004 and 2019 for hematologic malignancies (Acute lymphoblastic leukaemia, ALL, Hodgkin's lymphoma, HL, and non-Hodgkin's lymphoma, NHL).

Hyperglycaemia was defined as a random capillary blood or plasma glucose level exceeding 11 mmol/L (200 mg/dl) in at least two different measurements separated by 24 h. All patients received steroid therapy; asparaginase was prescribed to ALL (100%) and NHL patients (33%).

Hyperglycaemia occurred in 18% of ALL patients and 17% of NHL patients, mostly within the first month of treatment, corresponding to pre- and induction phases, and a reduced prevalence during maintenance and remission. The median follow-up duration of blood glucose monitoring was 8.6 months for ALL patients and 3.6 months for NHL patients. At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter. Half of ALL patients with hyperglycaemia were treated with insulin and required insulin therapy only during ALL treatment, except one patient who remained insulin-dependent after therapy completion. Obesity/overweight, ongoing puberty at the time of cancer diagnosis, the presence of steroid-resistant disease and the use of HSCT were associated with a higher risk of developing hyperglycaemia in ALL patients.

The study confirms a non-negligible prevalence of hyperglycaemia in patients treated for ALL. Strengths of the study are the large sample size of ALL patients and the evaluation of this possible complication from the start of cancer treatment and not only during remission. Possible limitations are the retrospective design and the small sample size of NHL and HL patients. Furthermore, glucose data in remission phase were unavailable in particular for HL patients. Anthropometric parameters, as waist-to height ratio or waist to hip ratio, considered as indicators of central (visceral) adiposity and metabolic risk in these patients, were not analyzed. Inpatients underwent daily blood tests, which periodically included measurement of plasma glucose levels. Clinical follow-up did not include oral glucose tolerance testing, and this may have led to an underestimation of the proportion of off-therapy patients with impaired glucose tolerance or preclinical diabetes mellitus.

9.4. Metabolic syndrome in male survivors of pediatric allogeneic hematopoietic stem cell transplantation: impact of total body irradiation, low-grade inflammation, and hypogonadism

Muhic E, Mathiesen S, Nielsen MM, Suominen A, Sørensen K, Ifversen M, Nolsöe RL, Pedersen KM, Lähteenmäki P, Nordestgaard BG, Juul A, Jahnukainen K, Müller K
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Transplant Cell Ther. 2021 Sep;27(9): 778.e1-778.e8.
PMID: 34091072.

Brief Summary: Several studies in cancer survivors have reported an increased prevalence of metabolic syndrome (MetS), which is often observed in the absence of overt obesity. Data about MetS in survivors of pediatric hematopoietic stem cell transplantation (HSCT) are scarce. This cross-sectional cohort study evaluated the prevalence and clinical presentation of MetS in 98 adult male survivors of pediatric HSCT (median follow-up 18 years, 74% received total body irradiation, TBI) and 4767 male controls from the background population.

MetS was diagnosed when three the following criteria were present: fasting plasma glucose > 5.6 mmol/L, HDL <1.03 mmol/L, fasting plasma triglyceride >1.7 mmol/L, blood pressure >130 mmHg systolic or >85 mmHg diastolic or treatment for these conditions, abdominal circumference >102 cm. The prevalence of MetS in HSCT survivors was 30% (vs 18% in age-matched controls), corresponding to the prevalence observed in 50- to 80-year-old males from the background population. Hyperglycemia was more common in HSCT survivors with MetS, in comparison with age-matched controls with MetS and 50- to 80-years old men from the reference population (76% vs 20% vs 39%). HSCT survivors with MetS had normal or low BMI more commonly than age-matched controls with MetS (41% vs 11%). MetS was associated with total body irradiation (TBI) conditioning regimen, lower testosterone levels, and higher levels of IL-6 and high sensitivity C-reactive protein. Fat distribution was evaluated by android/gynoid (AG) ratio from a whole-body dual-energy X-ray absorptiometry (DXA). Abdominal fat accumulation (increased AG fat ratio) was strongly associated with MetS, despite abdominal circumference was normal in most HSCT patients with MetS. TBI was associated with a higher abdominal adiposity, increased fasting glucose and lower testosterone levels.

This well-designed study reported an increased risk of MetS with a peculiar clinical picture, characterized by hyperglycemia and fat redistribution, with abdominal fat accumulation (despite a normal BMI) as driving factors. TBI is confirmed to be the most relevant risk factor for MetS, and there was no significant impact of primary diagnosis, age at HSCT, or graft-versus-host disease. It would have been interesting to evaluate, in addition to the abdominal circumference, the waist-to-hip and the waist-to-height ratios that represent simple parameters correlated with abdominal adiposity in cancer survivors, and can be related to DXA parameters. In fact, whole body DXA scan for body composition analysis is hardly feasible in all HSCT survivors due to its costs and radiation exposure.

Adrenal Function after Cancer Treatment

9.5. Adrenocortical function in children with brain tumors and pediatric hematopoietic cell transplantation recipients

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J Pediatr Hematol Oncol. 2022; 44: e469-e473.
PMID: 34054040.

Brief Summary: This monocentric, retrospective study investigated adrenal insufficiency (AI) prevalence in a large group of children with brain tumors (BT, 398 patients) and children treated with hematopoietic cell transplantation (HCT, 268 patients). AI was diagnosed in 14 BT patients (27.4% of the 51 tested) and 14 HCT recipients (21.2% of the 66 tested).

Children with BT and children undergoing HCT may develop central AI due to exogenous glucocorticoids, and/or direct damage of the hypothalamic-pituitary (HP) area induced by the tumor location itself, surgery, and/or radiotherapy. In this study, low-dose ACTH test (1 mcg cosyntropin intravenously) was used in cases of suspected central AI. High-dose ACTH test (15 mcg/kg cosyntropin intravenously, maximum 250 mcg) was performed to diagnose primary AI. In this study, the institutional medical records were searched between 2006 and 2017. ACTH stimulation testing was performed in 51/398 (12.8%) children with BT and in 66/268 (24.6%) HCT recipients, and AI was defined as peak serum cortisol <18 mcg/dL

AI was diagnosed in 14/51 (27.4%) of BT patients tested. There was a higher rate of AI in patients with direct involvement of the hypothalamic-pituitary-adrenal (HPA) axis by tumor (36% vs 21%), in those undergoing surgery involving the hypothalamic-pituitary (HP) region (50% vs 23%) and in those who received ≥30Gy dose of cranial radiotherapy (32% vs 11%). Maintenance and stress-dose glucocorticoids were recommended in 81% of patients with inadequate response at ACTH test, stress-dosing alone was recommended in 19%.

AI was diagnosed in 14/66 (21.2%) of HCT recipients tested. In this group, 9/66 patients had received TBI. The most common indication for testing (56% patients) was exogenous glucocorticoid exposure. The rate of AI was

higher in patients with graft-versus-host disease (36% vs 12.5%), who frequently receive exogenous steroids. Maintenance and stress-dose glucocorticoids were recommended in 80% of patients with inadequate response at ACTH test, while stress-dosing alone was indicated in 20%.

The prevalence of iatrogenic AI in children with cancer is probably underestimated and can be life-threatening. Among children with BT, AI is more often diagnosed in patients with primary lesion involving the HP region, in those who received cranial radiotherapy, and those with other pituitary deficiencies. These observations emphasize the importance of testing for AI all patients carrying these risk factors, in particular before any surgical procedure or general anesthesia, due to the risk of acute adrenal failure. For this purpose, a close collaboration between pediatric hematology-oncology and endocrinology specialists is advocated. Strengths of the study are the large sample of BT and HCT patients. In this study, only a minority of patients underwent ACTH testing so possibly many other cases were missed. Other limitations are its retrospective nature and the lack of repeated tests to distinguish patients who had temporary AI due HPA axis suppression related to prolonged steroid treatment.

The most common etiology of AI was adrenal suppression from exogenous glucocorticoids. One patient had primary AI related to MIRAGE syndrome, and another patient developed primary AI following bilateral adrenalectomy for neuroblastoma.

Fertility Issues and Reproductive Outcomes in Childhood Cancer Survivors

9.6. Serum anti-müllerian hormone levels and risk of premature ovarian insufficiency in female childhood cancer survivors: systematic review and network meta-analysis

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Cancers 2021, 13, 6331.
PMID: 34944951.

Brief Summary: This systematic review and meta-analysis examined the value of anti-Müllerian hormone (AMH) to predict premature ovarian failure (POI) in childhood cancer survivors (CCSs), compared to the general population.

The potential toxic effect of alkylating agents and body irradiation on female reproductive function is well known. To date, antral follicle count (AFC) by transvaginal ultrasound is considered the most reliable measurement to predict ovarian reserve. AFG is not applicable in young children and is often refused by adolescents due to discomfort. Several studies aimed to identify a specific biochemical marker to predict POI. AMH is secreted by granulosa cells of growing pre- and early antral ovarian follicles. The serum level of AMH is low during childhood, increases during puberty reaching a plateau during the mid-twenties; thereafter it decreases until the menopause. Age-related reference ranges are available. AMH is representative of ovarian reserve in all ages and its level is not dependent on the menstrual cycle phase.

The Authors identified 251 studies, but selected only the 8 studies which had an appropriate age-matched control group (1303 participants: 663 CCSs and 640 healthy controls). A forest plot and a prediction interval plot were constructed to compare the impact of the different cancers on women's fertility (AMH levels) and to rank them using a ranking plot (Surface Under the Cumulative Ranking curve Area (SUCRA). The SUCRA is a numeric representation of each treatment effect by a percentage, ranging from 0 to 100%. The closer the SUCRA is to 100%, the higher is the likelihood that a therapy ranks as the most influential.

Women treated for a neuroblastoma during infancy ranked first for impaired AMH levels (SUCRA 65.4%), followed by mixed CCSs (29.6%). Neuroblastoma survivors showed the highest rates of POI (42.5%), followed by acute lymphoid leukaemia (26.3%) or any other neoplasia (20.5%), and rare chances were reported in patients with thyroid cancer (1.9%) or no cancer (1.6%).

The Authors concluded that AMH levels represent a reliable indirect tool to predict POI. The major strength of this meta-analysis is that all the 8 selected studies included a matched control group. On the other hand, the

selected studies had been performed between 1964 and 2017, on patients affected by different neoplasms treated with various therapeutic regimens, some of which are now outdated.

9.7. Fertility status among long-term childhood acute lymphoblastic leukaemia survivors enrolled between 1971 and 1998 in EORTC CLG studies: results of the 58 late adverse effects study

G. Rossi, M. Kicinski, S. Suciu, E. Vandecruys, G. Plat, A. Uyttebroeck, C. Paillard, M. Barbati, M.F. Dresse, P. Simon, O. Minckes, C. Pluchart, A. Ferster, C. Freycon, F. Millot, J. van der Werfften Bosch, C. Chantrain, R. Paulus, T. de Rojas, G. de Schaetzen, P. Rohrlich, Y. Benoit, C. Piette

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Human Reproduction, 2022; 37: 44–53.

PMID: 34788455.

Brief Summary: This case-control study evaluated fertility status in childhood acute lymphoblastic leukaemia (ALL) survivors enrolled in the European Organisation for Research and Treatment of Cancer (EORTC) Children's Leukemia Group (CLG) Late Adverse Effect (LAE) study. The enrolled patients had been treated for ALL between 1971 and 1998 with different protocols. Both patients and controls completed a questionnaire about fertility and parenthood. The results showed more common pregnancy problems among female survivors aged $\geq$10 years at ALL diagnosis, and higher risk of miscarriages among partners of male survivors. These findings were related to the damage induced by cranial radiotherapy (CRT) on the function of the hypothalamic-pituitary-gonadal axis, and to the effect of alkylating agents in inducing germline mutations.

The recent PanCareLIFE Consortium guidelines (1, 2), based on current childhood ALL treatment protocols (HSCT, low-dose alkylating agents (cyclophosphamide-equivalent dose $<$6000–8000mg/m2) and CRT), suggest that oocyte or embryo cryopreservation should be recommended before HSCT in post-pubertal female ALL patients. The same measures should be suggested in patients at high recurrence risk, before low-dose alkylating agents or CRT. Ovarian tissue cryopreservation should be suggested in pre-pubertal female ALL patients before HSCT. Sperm cryopreservation should be recommended before HSCT or testicular radiotherapy, low-dose alkylating agents or CRT in post-pubertal male ALL patients. In pre-pubertal male ALL patients, testicular tissue cryopreservation should be suggested before HSCT and testicular radiotherapy. The Authors underlined that the high percentage of fertility problems among controls could have affected the results. However, the large study group of patients affected by the same malignancy and the analysis of males and females separately add relevance to the results, which are very encouraging for ALL patients. One-to-one controls were matched by region, level of urbanization, sex and education, and this is undoubtedly the strongest point of the study. Nonetheless, other similar studies are needed in the near future, in view of the change in therapeutic approach to this malignancies (i.e. chimeric antigen receptor T-cells), that is now modifying the scenario of late adverse effects in ALL survivors.

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9.8. Temporal changes in the probability of live birth among female survivors of childhood cancer: a population-based adult life after childhood cancer in Scandinavia (ALiCCS) study in five Nordic countries

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Cancer 2021; 127: 3881-3892.
PMID: 34297360.

Brief Summary: This register-based cohort study analysed the likelihood of live birth among female childhood cancer survivors (CCSs) diagnosed in between 1943 and 2006, in comparison with the general population. The prevalence of first live birth was lower in CCSs compared to matched controls (63% versus 80.9%).

Patients were identified using the unique population-based Nordic registries of Denmark, Finland, Iceland, Norway and Sweden, collecting data about 8,886 CCSs and 62,903 age- and country-matched controls, for a long follow-up. For every CCSs, data about radiotherapy (RT) were categorized according to target organ (uterus, ovaries or pituitary) and estimated radiation dose (low, low-medium, medium-high, high or unknown). Unfortunately, in this work data about chemotherapeutic regimen or hematopoietic stem cell transplantation were lacking, so the concomitant impact of these factors on fertility was not analysed.

First live birth from age 15 years or for 5 years after cancer diagnosis were analysed by cancer type, age at cancer diagnosis, radiation status and dose and treatment period. The results confirmed the reduction in first live birth in CCSs compared to matched controls (63% versus 80.9%); after adjustment for country and maternal birth year, CCSs had 21% lower probability of livebirth than the general population at every maternal age. Malignancies with more reduced probability of livebirth were germ cell, central nervous system and renal tumours, probably reflecting a more extensive RT use in these malignancies.

The relative probability of a first live birth in CCSs increased with time, and the same trend was seen in all maternal age groups. Among women whose cancer was diagnosed between 1954 and 1989, the probability of a live birth at age 30 years was 31% to 35% lower than in controls, whereas it was only 9% lower for CCSs diagnosed between 1990 and 1999 and 6% lower in survivors of cancers diagnosed between 2000 and 2006. On the contrary, cumulative incidence of a first live birth did not change in the general population during the decades, confirming that the main determinant of this improvement was not a variation of background rates, but a significant improvement of treatment protocols and attention on fertility issues, in particular for patients receiving RT.

These improving trends with time are reassuring and underline the importance of focusing the future research on specific fertility risk in relation to cancer type and treatment protocols. Female patients with childhood cancer should receive personalized counselling regarding their specific infertility risk and fertility preservation options, in order to make informed decisions about future family planning.

9.9. Parenthood among men diagnosed with cancer in childhood and early adulthood: trends over time in a Danish national cohort

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Human Reproduction, Vol.36, No.9, pp. 2576–2586, 2021.
PMID: 34166497.

Brief Summary: This Danish register-based cohort study investigated the rate of fatherhood among 9,353 men diagnosed with cancer during childhood or early adulthood (CSs), compared with 1,386,493 age-matched males without cancer. CSs became fathers slightly less frequently than controls (36% versus 42%).

CSs were divided according to diagnosis (central nervous system, CNS, tumours, haematological cancer or solid tumours), age at diagnosis/study entry (<9, 10-19 and 20-29 years) and year of diagnosis/study entry (4 decades, from 1978 to 2016). Survivors became fathers less frequently than controls (36% versus 42%); in particular, fatherhood least frequent in survivors of CNS cancer (hazard ratio (HR) = 0.67) and highest in solid cancer survivors (1.16). The outcome in CNS survivors was correlated with a higher rate of adverse effects (clinical, psychological and psychosocial) related to both cancer specific characteristics and its treatment. Fatherhood in lymphoma survivors was higher than in leukaemia survivors (HR 0.92 versus 0.71), probably due to different therapeutic strategies, but unfortunately this data was lacking. Moreover, HR increased in patients

with higher age at cancer diagnosis and in patients diagnosed in recent decades. The trend in improvement of HR over time was confirmed in all cancers, and it was already evident shortly after diagnosis.

In the last few years many techniques of cryopreservation (semen for post-pubertal patients, testicular tissue for pre-pubertal patients) have been developed and the fatherhood desire represents now an important issue to be discussed at the moment of cancer diagnosis.

These results are encouraging in comparison with previous reports. Time trends indicate that a higher proportion of men with a cancer diagnosis during childhood are now able to become fathers, confirming that their life conditions have improved over time. A multidisciplinary team involving fertility specialists and oncologists should be involved in the evaluation of fertility risk at cancer diagnosis and during follow up, in order to ensure and prioritise individualized fertility counselling.

9.10. In male Hodgkin lymphoma patients, impaired fertility may be improved by non-gonadotoxic therapy

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British Journal of Haematology, 2022, 196, 110–115.
PMID: 34462914.

Brief Summary: This prospective single-centre study evaluated fertility in men diagnosed with Hodgkin lymphoma (HL) by sperm analysis, in order to identify clinical and biological characteristics correlated with fertility status. Only 28% of men showed normal sperm analysis.

Previous studies in patients with HL reported reduced fertility rates already present at diagnosis and prior to any treatment, indicating that HL-associated factors may contribute to infertility (1- 3). In addition, it is well known that high-dose of alkylating agents are responsible of germ cell damage at several steps of the differentiation and maturation of these cells.

This study included 100 HL patients diagnosed between 2008 and 2016. Median age at HL diagnosis was 25 years (range 15–37). After counselling on fertility preservation, 46 patients requested semen cryopreservation and 54 refused it; follow-up data was available for only 24 patients. All patients were treated with first-line chemotherapy (ABVD: adriamycin, bleomycin, vinblastine and dicarbazine), with or without radiotherapy (RT), followed by salvage chemotherapy and stem cell transplantation (HSCT) in case of refractory HL or relapse.

Pre-treatment HL patients had decreased fertility parameters compared to healthy controls, in terms of median sperm concentration and median percentages of normal-motility and normal-morphology sperms. Severe damaged sperm was observed in 17% of cases, while only 28% of patients showed normal sperm analysis. Regarding the prognostic factors, disease stages III and IV were associated with reduction in the number of spermatozoa, their motility and vitality. 54 months after completion of HL treatment, sperm motility was lower in HL patients who received HSCT, confirming that HSCT is associated with reduced spermatogenesis recovery and the most severe fertility damage.

Although this study was conducted on a small sample size of young-adult patients, it gives us interesting information about fertility status in HL, confirming the presence of specific HL-associated alterations before treatment. This could be useful not only for tailored fertility counselling, but also for improving cryopreservation technique, which have to take in account a pre-existing damage of germ cells.

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9.11. Assessment of the architecture and integrity of frozen-thawed testicular tissue from (pre)pubertal boys with cancer

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Andrology. 2022; 10: 279–290.
PMID: 34628730.

Brief Summary: This multicentric prospective study performed an accurate evaluation of testicular tissue after frozen-thawing to investigate if this testicular tissue freezing (TTF) alters testicular architecture and integrity in pre-pubertal and pubertal boys with cancer. Promising histological outcomes were found.

Spermatogonial stem cells (SSCs) are the testicular cells most sensitive to chemo- and radiotherapy-induced damage. Sperm cryopreservation is easily performed in adolescents and young adults. Testicular tissue cryopreservation is more difficult in pre-pubertal and pubertal boys. Currently, testicular tissue freezing (TTF) is suggested before cancer treatment, especially in young patients with high-risk of fertility impairment, but there is very little data on the histological assessment and quality of (pre)pubertal testicular tissue before and after thawing. This study explored the impact on human testicular tissue of a controlled slow freezing (CSF) protocol without seeding previously developed in mice (1, 2).

This study included 87 boys (aged 6 months-16 years) with cancer diagnosis and relapse after remission or incomplete response to the initial treatment. All patients had received chemotherapy before TTF and were candidates to highly sterilizing treatments (i.e. conditioning for HSCT with either total body irradiation or myeloablative chemotherapy).

The most interesting result is that histological analysis conducted in 74 patients revealed that CSF without seeding technique was able to maintain seminiferous tubule architecture, integrity and concentration of spermatogonia, and expression of DNA replication and repair marker in spermatogonia and Sertoli cells. The global "cryodamage score" did not vary significantly according to patient's age, pubertal stage or cancer types. Testicular tissue abnormalities were rare and similarly found on fresh tissue or after thawing, suggesting that they were generated by previous cancer treatment.

The capacity of human spermatogonia to differentiate after thawing was not analysed. The Authors had previously studied differentiation using testicular tissue grafting and *in vitro* maturation in mice. Sperm production was demonstrated in testicular tissue cryopreserved without seeding after allograft into nude mice or after *in vitro* spermatogenesis. Future research is needed to add clinical relevance to this promising technique confirming its feasibility and reliability in humans.

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9.12. Pregnancy and pregnancy outcomes after hematopoietic stem cell transplantation in childhood: a cross-sectional survey of the EBMT pediatric diseases working party

Diesch-Furlanetto T, Rovó A, Galimard JE, Szinnai G, Dalissier A, Sedlacek P, Bodova I, Roussou VK, Gibson BE, Poiré X, Fagioli F, Pichler H, Faraci M, Gumy-Pause FG, Dalle JH, Balduzzi A, Bader P, Corbacioglu S
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Hum Reprod. 2021; 36: 2871-82.
PMID: 34529796.

Brief Summary: This retrospective study of the European Society for Blood and Marrow Transplantation (EBMT) registry describes the natural or assisted conceptions and their outcomes in patients $<$18-year-old at their first transplantation who received hematopoietic stem cell transplantation (HSCT) between 1995 and 2016.

In total, 62,988 pediatric patients received a first HSCT in EBMT centers. Pregnancy was reported in 406 patients in the database. Details concerning the first pregnancy and pregnancy outcome were obtained using a questionnaire from 99 patients (24%). Of the 99 patients, 29 (29%) were men who reported a pregnancy of their partners. All the seven men who had received conditioning regimens with TBI achieved fatherhood but required assisted fertilization (5/7) or used their cryopreserved sperm (2/7). The median age at conception in women was 25.0 years, about 5 years lower than the age of healthy women at their first child's birth, 52% of women conditioned with total body irradiation (TBI) and 96% of those conditioned without TBI conceived naturally. 90% of all conceptions ended in a live birth, 84.5% were at term and 93% had normal birthweight.

Conditioning regimens before HSCT are highly gonadotoxic, which leads to gonadal failure and pubertal development disorders. There are few population-based studies assessing the risk of future infertility in children after HSCT. This study highlights that conception after HSCT, even after myeloablative conditioning, is possible, even if the support of reproductive medicine is required in several cases. A limitation of this study is that the age at last follow-up was <17 years for 75% of patients in the EBMT pediatric dataset, therefore a more prolonged follow-up is needed to allow all patients to realize their reproductive potential and adequately estimate the cumulative incidence of conception.

Timely discussion and counseling regarding possible late effects of cancer treatment are important to support patients' future family planning. Whenever possible, fertility preservation options should be illustrated to parents of children and adolescents at cancer diagnosis. The direct toxicity caused by alkylating agents and radiation on the ovaries severely reduces follicular reserve inducing premature menopause. This study highlights that, even after HSCT, there may be a "time window of opportunity" when fertility is preserved for conceiving naturally or with the support of reproductive medicine. A transient recovery of the ovarian function after a few year from HSCT had already been reported, mostly in women <25 years old, but the identification of these patients at the right time remains a challenge (1). Children should be followed regularly after cancer treatment to allow patients who could not benefit from fertility preservation options before HSCT to take advantage of the 'fertility window of opportunity' afterwards.

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9.13. Serum anti-Müllerian hormone as a marker of ovarian reserve after cancer treatment and/or hematopoietic stem cell transplantation in childhood: proposal for a systematic approach to gonadal assessment

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Eur J Endocrinol. 2021; 185: 717-728.

PMID: 34519276.

Brief Summary: This retrospective study, involving two Italian centers, enrolled post-pubertal female patients who had been treated with gonadotoxic therapies for hematological malignancies and/or hematopoietic stem cell transplantation (HSCT) before the age of 18 years.

Several studies have reported the association between therapy with alkylating agents and ovarian failure but information is still lacking about the continuous relationship between increasing doses of antineoplastic drugs and reduced anti-Müllerian hormone (AMH) levels as an index of impaired ovarian reserve. The severity of gonadotoxicity depends on the cumulative burden of chemotherapy and a specific algorithm has been proposed to normalize the dose of any alkylating agent into the corresponding cyclophosphamide equivalent dose (CED), considered as a reliable tool to quantify the total exposure to gonadotoxic treatments and compare different antineoplastic protocols (1).

The aim of study was to analyze the pattern of residual ovarian function and assess the relationship between cyclophosphamide equivalent dose (CED) and anti-Müllerian hormone (AMH). Based on the analysis of the

collected data, a systematic algorithm was developed to quantify iatrogenic gonadal impairment according to AMH levels. According to previous data on ovarian reserve in childhood cancer survivors AMH-SDS ≥ -1.65 was considered normal, whereas values < -1.65 were classified as 'low'. Ovarian reserve was defined as:

- normal ovarian reserve (NOR): regular menses, AMH SDS ≥ -1.65 and normal FSH levels
- diminished ovarian reserve (DOR): AMH-SDS < -1.65, despite normal gonadotropins (FSH $\leq$ 25 UI/L) and regular menses.
- premature ovarian insufficiency (POI): 4–6 months of oligo-amenorrhea associated with FSH $>$25 IU/L at two consecutive measurements recorded more than 4 weeks apart.

POI was diagnosed in 72.1% of women treated with HSCT and in 3.7% of non-HSCT women. DOR was present in 16.3% of women treated with HSCT and 22.2% of non-HSCT women. Conditioning regimens played a key role on ovarian outcome in HSCT patients; 100% of either total body irradiation (TBI) or busulfan-exposed patients presented ovarian impairment, while patients treated with cyclophosphamide developed POI in 44% of cases, DOR in 22%, and showed a normal ovarian reserve in the remaining cases. Similarly, AMH levels were higher amongst patients who received cyclophosphamide compared with those who received busulfan or TBI-based conditionings. Higher CED values were associated with lower AMH-SDS, with the value of 7200 g/m2 discriminating the best cut-off between DOR/POI and normal ovarian function. Age at cancer diagnosis $\geq$10 years negatively affected ovarian reserve.

This study confirms that radiotherapy, older age at diagnosis, and HSCT increase the likelihood of developing ovarian impairment and support the recommendation for ovarian assessment for all women treated with alkylating agents and/or radiotherapy, in particular those exposed to CED $\geq$ 7200 mg/m2. High gonadotropin levels and oligo-amenorrhea are still routinely used to diagnose POI, but an integrated evaluation of AMH levels, age at chemotherapy exposure, and an adequate assessment of the overall treatment-related burden could guide clinicians in providing patients with personalized counselling about fertility. The early identification of patients with ovarian reserve impairment, could lead to a prompt undertaking of fertility preservation techniques by exploiting the "opportunity window" preceding the progression into overt POI.

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9.14. Effect of Genetic Variation in CYP450 on gonadal impairment in a European cohort of female childhood cancer survivors, based on a candidate gene approach: results from the PanCareLIFE study

van der Perk MEM, Broer L, Yasui Y, Robison LL, Hudson MM, Laven JSE, van der Pal HJ, Tissing WJE, Versluys B, Bresters D, Kaspers GJL, de Vries ACH, Lambalk CB, Overbeek A, Loonen JJ, Beerendonk CCM, Byrne J, Berger C, Clemens E, Dirksen U, Falck Winther J, Fosså SD, Grabow D, Muraca M, Kaiser M, Kepák T, Kruseova J, Modan-Moses D, Spix C, Zolk O, Kaatsch P, Krijthe JH, Kremer LCM, Brooke RJ, Baedke JL, van Schaik RHN, van den Anker JN, Uitterlinden AG, Bos AME, van Leeuwen FE, van Dulmen-den Broeder E, van der Kooi ALF, van den Heuvel-Eibrink MM

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Cancers (Basel). 2021; 13: 4598.

PMID: 34572825.

Brief Summary: This cohort study of female childhood cancer survivors (CCS) identified associations between specific nucleotide polymorphisms (SNP) in cytochrome P450 (CYP450) enzymes, which metabolize alkylating agents (AA), and variability in AA-induced ovarian damage.

Chemotherapy with alkylating agents (AA) is a well-recognized risk factor of gonadal failure in female childhood cancer survivors (CCS). However, significant inter-individual variability in ovarian damage is reported and polymorphisms in the genes encoding these enzymes may explain this variability.

Anti-Müllerian hormone (AMH) levels served as a proxy for ovarian function in a discovery group of adult female CCS, from the pan-European PanCareLIFE cohort (discovery cohort). The results were validated, and meta-analysis performed, using the USA-based St. Jude Lifetime Cohort (replication cohort). Eligible participants were women diagnosed with cancer before the age of 25 years and treated with chemotherapy. They had survived at least 5 years after diagnosis and were >18 years of age at evaluation. Nine genetic variants in three CYP450 enzymes were analyzed in relation to cyclophosphamide equivalent dose (CED) and its impact on AMH levels.

The polymorphism CYP3A4*3 was associated with lower AMH levels in both the discovery and replication cohort. Meta-analysis showed a deleterious effect of CYP3A4*3 on AMH levels. Conversely, the polymorphism CYP2B6*2 showed a protective effect on AMH levels in CCS receiving a CED >8000 mg/m^2.

Single nucleotide polymorphisms (SNPs) in CYP genes have been associated with cyclophosphamide toxic effects on ovaries in exposed adult women (1). This is the first study to evaluate the effect of SNPs in CYP450 enzymes on gonadotoxicity due to AA exposure during childhood. The results may improve clinical practice by identifying patients at high risk of ovarian impairment who may benefit from early referral and counseling about fertility preservation options. For young girls at high risk of infertility, preservation measures involve the surgical removal of an ovary (or part of it) with ovarian tissue cryopreservation. Cryopreservation of oocytes is only possible in some older adolescent patients, who can postpone cancer treatment. Pharmacogenetic is increasingly used to personalize therapy and minimize side effects. In the near future, evidence-based knowledge of genetic predisposition to AA toxicity may optimize the delivery of tailored therapy and fertility counselling. Future prospective research is important to define the clinical relevance of polymorphisms in determining the gonadotoxic effect of cancer treatment. Moreover, differences in pharmacokinetics between children and adults may be potentially involved and require further study.

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Bone Health in Chronic Disease

9.15. Bone health in pediatric patients with Crohn disease

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J Pediatr Gastroenterol Nutr. 2021; 73: 231-235.
PMID: 33908740.

Brief Summary: This retrospective study evaluated longitudinal changes in bone mineral density (BMD) in children and adolescents with Crohn disease (CD), and the risk factors for low BMD. Low BMD (defined as BMD Z score $\leq$ -2.0) was present in 18.7% of patients at diagnosis and in 16% at the end of follow up.

193 children with CD, aged 2 to 18 years, underwent dual-energy X-ray absorptiometry (DXA) both at diagnosis and at the end of follow-up between 1999 and 2018. Data were checked using two different sources (a medico-administrative database and a national register).

Lumbar spine (LS) BMD values were lower than total body less head (TBLH) values, both at diagnosis and at the end of follow-up. Multivariate analysis showed that height growth impairment or low body mass index (BMI) were associated with low BMD at diagnosis, while at the end of follow-up only cumulative steroid dose was associated with low BMD.

The large sample size and the double source of data are strengths, but its retrospective design and the lack of some relevant data, such as pubertal status, physical activity, diet, and calcium/vitamin D intakes limits its significance. Another limitation is related to the use of DXA. Although DXA is still considered the most

appropriate method for BMD assessment, this technique analyzes bone mineral content by area and not by volume, not considering the three-dimensional structure of the bone. Thus, DXA intrinsically underestimates BMD of children with linear growth delay.

Low BMD is the most common extra-intestinal manifestation (from 6 to 44%) after inflammatory bowel disease (IBD) diagnosis. This study confirms that long-term glucocorticoid therapy is the main risk factor associated with low BMD. Special attention must be given to patients with height growth delay and/or low BMI at diagnosis. As the authors suggest, an age-appropriate counseling for nutrition and physical activity, may empower young patients and their families to prevent calcium/vitamin D deficiency and decreased skeletal muscle mass, which can contribute to poor bone health in IBD.

9.16. Skeletal adverse events in childhood cancer survivors: An adult life after childhood cancer in Scandinavia cohort study

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Int J Cancer. 2021; 149: 1863-1876.
PMID: 34278568.

Brief Summary: This population-based study retrospectively compared hospital admissions for skeletal issues in a group of 26,334 cancer survivors (CCS) diagnosed before 20 years of age and 127,531 matched controls. 1,987 CCS vs 8,986 controls had at least one skeletal adverse event. Total hospitalization rate ratio (RR) for skeletal adverse events was higher in CCS than controls (RR 1.35).

RR for hospitalization for skeletal adverse events was increased for osteonecrosis (RR 25.9), osteoporosis (RR 4.53), fractures (RR 1.27), osteochondropathies (RR 1.57) and osteoarthrosis (RR 1.48). The RR for any skeletal adverse event in CCS was highest for the first 5 years from diagnosis (RR 1.6) and remained higher than in the general population up to age 60 years. 43% of events occurred before age 20 years, more commonly in males (8.6% vs 6.3% of females) and for the following cancer diagnoses: malignant bone tumors (RR 2.6), sympathetic nervous system tumors (RR 1.7), and leukaemia (RR 1.6). The RR of osteoporosis was higher in CCS who were 0-9 years of age at cancer diagnosis, and in patients with leukemia (RR 28.2). The RR of fractures in CCS was more elevated for serious fractures (hip RR 2.9; femur RR 2.0), than for osteoporotic fractures (proximal humerus, distal radius, distal femur, proximal tibia, pelvis and vertebrae, RR 1.4). The patterns of hospitalizations for fractures were similar among survivors and control subjects, but the expected age-dependent increase in the hospitalization rate occurred significantly earlier among cancer survivors.

An increased risk of low bone mineral density during and early after the completion of childhood cancer treatments has been reported repeatedly, but data on long-term effects and fracture risk are still conflicting. Among the strengths of this study, differently from studies based on questionnaires, skeletal adverse events were medically verified and their registration was mandatory for treating physicians. Data were cross-checked using national cancer registries and national population registries (precious resources available in the Scandinavian countries). A limitation was the lack of treatment data, in particular glucocorticoid exposure, well-known risk factor for both osteoporosis and osteonecrosis. Similarly, information about vitamin D status, nutrition, dietary calcium intake and daily physical activity was unavailable.

10. Type 1 Diabetes

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Reviews

10.1. A century past the discovery of insulin: global progress and challenges for type 1 diabetes among children and adolescents in low-income and middle-income countries

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Lancet. 2021 Nov 13;398(10313):1837-1850.
https://pubmed.ncbi.nlm.nih.gov/34774146/

Brief Summary: This review summarizes key aspects of type 1 diabetes (T1D), such as epidemiology, pathogenesis, risk factors and management. While reviewing and discussing these topics, the central theme of the review is the burden of T1D and the challenges encountered in its daily management in low- and middle-income countries (LMICs).

2021 was a special year for T1D, marking the centenary of the discovery of insulin. Several papers were published in 2021-2022 reviewing the enormous progresses made in the last 100 years in the field of insulin therapy (1,2). Whilst celebrating these achievements, reflections on global disparities in the management of T1D cannot be ignored. The burden of T1D among children and adolescents remains disproportionate in LMICs. This is due to lack of early detection and diagnosis of this condition and related comorbidities and complications, due to limited and inappropriate infrastructures, diagnostic and management capacities. Alarmingly, only 20% of countries in central and south Asia, Mali, Mozambique have access to insulin. Access to technology, which has revolutionized the treatment of this condition in high income countries, is still limited in LMICs.

The incidence of T1D is increasing worldwide, but there is lack of epidemiological data from LMICs. Studies looking at the pathogenesis and risk factors for T1D primarily include populations from high income countries, therefore limiting the understanding of the effect of specific environmental or socio-demographic factors in LMICs. The Covid-19 pandemic further accentuated some of the issues in LMICs, with disruptions in supplies of insulin, glucose strips and need to rationalize their use due to increased costs.

Therefore, there is a need of clear plans and more investments to improve access to insulin and essential technologies to improve the diagnosis and management of T1D in young people living in LMICs.

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10.2. Screening for type 1 diabetes in the general population: a status report and perspective

Sims EK, Besser REJ, Dayan C, Geno Rasmussen C, Greenbaum C, Griffin KJ, Hagopian W, Knip M, Long AE, Martin F, Mathieu C, Rewers M, Steck AK, Wentworth JM, Rich SS, Kordonouri O, Ziegler AG, Herold KC
NIDDK Type 1 Diabetes TrialNet Study Group.
Diabetes. 2022 Apr 1;71(4):610-623.
https://pubmed.ncbi.nlm.nih.gov/35316839/

Brief Summary: This is a comprehensive review on general population screening, a current hot topic in the field of type 1 diabetes (T1D). The authors provide a critical overview of the rationale for population screening, arguments for and against it, current efforts to guide this program and the key hurdles to address.

The introduction of a general population screening for T1D offers an appealing opportunity to shift the diagnosis at an early asymptomatic stage and reduce the burden associated with T1D. An early identification of children at risk of T1D can prevent severe acute presentations such as diabetic ketoacidosis (DKA), whose prevalence is still unacceptably high, and is associated with substantial morbidity and mortality (1,2). There is optimism that in the future at-risk children could be offered treatment to delay or even prevent the onset of T1D. The recent anti-CD3 (teplizumab) trial showed the promise of immunotherapy in people at risk of T1D, with a single 14-day treatment course able to delay the diagnosis by an average of 2 years (2).

Advances in the understanding of the natural history of T1D has led to the characterization of pre-symptomatic stages (1 and 2) preceding clinical manifestations (stage 3). T1D-specific autoantibodies can be detected several years before the onset of the disease, and children with 2 or more autoantibodies have a risk of 84% of developing T1D over 15 years, supporting the use of autoantibodies as a useful screening tool (3). Screening programs so far have focused on relatives of people with T1D, who have a 15-fold increased risk of developing this condition compared to the general population. However, at least 85% of children with T1D do not have an affected family member, thus supporting the value of a general population screening.

The review addresses other key aspects, such as when to start screening and the most cost-effective approach. It also highlights the importance of providing the right education and psychological support to families. Follow up of children identified at risk needs to be defined. Partnership with community primary care providers will be essential for the implementation of general population screening.

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Clinical Trials

10.3. Randomized trial of closed-loop control in very young children with type 1 diabetes

Ware J, Allen JM, Boughton CK, Wilinska ME, Hartnell S, Thankamony A, de Beaufort C, Schierloh U, Fröhlich-Reiterer E, Mader JK, Kapellen TM, Rami-Merhar B, Tauschmann M, Nagl K, Hofer SE, Campbell FM, Yong J, Hood KK, Lawton J, Roze S, Sibayan J, Bocchino LE, Kollman C, Hovorka R, KidsAP Consortium

N Engl J Med. 2022 Jan 20;386(3):209-219.
https://pubmed.ncbi.nlm.nih.gov/35045227/

Brief Summary: This multicenter, randomized, crossover trial in 74 very young children (age: 1-7 years) with type 1 diabetes (T1D) tested the safety and efficacy of a hybrid closed-loop system for insulin delivery (CamAPS FX) compared with sensor-augmented pump therapy over 16 weeks. The hybrid closed-loop system showed better glycemic outcomes, without any difference in hypoglycemic episodes or adverse events.

Hybrid closed-loop systems, combining an insulin pump, a continuous glucose monitor (CGM) and a control algorithm, which automatically adjust basal insulin delivery based on glucose levels, are becoming progressively more available for the management of T1D. These systems have proven to improve glycemic control in older children and adults (1). However, there are limited data in younger children, a population where diabetes management can be challenging due to several factors, such as unpredictable eating and activity levels, variable insulin requirements and pronounced insulin sensitivity (2). This population is particularly vulnerable to the effect of hypoglycemia, due to lack of recognition of signs and symptoms. Greater frequency and severity

of hypoglycemia in this age group is associated with parental emotional distress and burden. Both hypoglycemia and prolonged hyperglycemia can have negative neurocognitive effects in young children (3).

This study shows a clear benefit associated with the use of hybrid closed loop system in this young population, providing reduced hyperglycemia, better time spent within target, without increasing the time spent in hypoglycemia. This supports the longer-term use of this system in this age group to improve glycemic control and hopefully reduce parental burden related to diabetes management. However, there are still some barriers to address before a wider implementation of this system into clinical practice, including associated costs and equitable access. In addition, adequate resources need to be in place to fulfil the required training for staff and patients/families, which is essential to achieve the best outcomes with hybrid closed-loop therapy.

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Mechanisms

10.4. Childhood body size directly increases type 1 diabetes risk based on a lifecourse Mendelian randomization approach

Richardson TG, Crouch DJM, Power GM, Morales-Berstein F, Hazelwood E, Fang S, Cho Y, Inshaw JRJ, Robertson CC, Sidore C, Cucca F, Rich SS, Todd JA, Davey Smith G

Nat Commun. 2022 Apr 28;13(1):2337.
https://pubmed.ncbi.nlm.nih.gov/35484151/

Brief Summary: This Mendelian randomization study analysed genetic data from 454,023 individuals from the UK Biobank and 15,573 type 1 diabetes (T1D) cases from other cohorts and provides strong evidence that larger childhood body size increases T1D risk, independently from body size at birth and during adulthood.

Observational studies have suggested a contributing role of obesity to the increasing rates of T1D (1). Several hypotheses have been formulated to explain this association, such as the 'accelerator hypothesis', which proposes that increased insulin resistance and insulin demand, related to excess body fat, cause beta cell stress and fragility, apoptosis and early autoimmunity (2). In addition, diets high in fat and carbohydrate and low in fibre can affect the metabolic and immune functions of the gut microbiome, which in turns has been linked to T1D risk (2). However, observational studies are limited in explaining causality because of possible bias from unmeasured confounding factors and reverse causation. Mendelian randomization is an approach using instrumental variable analysis, where genetic variants are used as a non-confounded proxy for the exposure of interest.

This study provides strong support for a link between childhood adiposity and T1D risk. The study also addressed the relationship between childhood obesity and other immune-mediated conditions, such as asthma, eczema and hypothyroidism, to explore if there was a generalizable effect of childhood adiposity on the immune system. Childhood obesity increases risk of these other diseases, but this is likely related to being overweight for many years, given that the effect was attenuated after accounting for adulthood body size.

Although the underlying mechanisms for the association between adiposity and T1D are not fully clear, these data support the existence of a critical window in childhood to mitigate the influence of adiposity on T1D.

These findings, along with previous observational data, strongly emphasize the importance of preventive measures to reduce the related global epidemics of childhood obesity and T1D.

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New Paradigms

10.5. Heterogeneity of type 1 diabetes at diagnosis supports existence of age-related endotypes

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Diabetes Care. 2022 Apr 1;45(4):871-879.

https://pubmed.ncbi.nlm.nih.gov/35147706/

Brief Summary: This Finnish cross-sectional register-based study of 6015 youth with new onset type 1 diabetes (T1D) explored potential differences in clinical, autoimmune and genetic characteristics across three age groups: <7, 7-12 and ≥ 13 years. Significant differences emerged primarily between the youngest and oldest age groups, confirming a substantial T1D heterogeneity associated with age at diagnosis.

Previous studies have highlighted the heterogeneity of T1D and introduced the important concept of age-related 'endotypes', which are disease subtypes defined by distinct pathophysiological mechanisms (1). Substantial differences were previously identified between children diagnosed <7 years *vs* ≥ 13 years. The former group was characterized by a hyperimmune pattern of insulitis, less residual insulin-containing islets, abnormal proinsulin processing and lower circulating C-peptide (2,3).

The present study explores differences in clinical and biochemical characteristics across different age groups, using a large population of Finnish youth newly diagnosed with T1D. The study confirms clear differences in children based on their age at diagnosis, particularly between the youngest and oldest age groups, with intermediate characteristics for the age group 7-12 years. Children diagnosed at age <7 years had a higher prevalence of affected first-degree relatives, stronger at-risk HLA, and higher number of autoantibodies than older children. This supports a more aggressive autoimmune process. This age group also showed shorter duration of symptoms and metabolic decompensation, likely reflecting higher awareness and recognition of early symptoms, which could be partly influenced by the higher prevalence of affected family members. Those diagnosed when ≥ 13 years had higher male preponderance, frequency of GAD autoantibodies, longer duration of symptoms before diagnosis, and more severe metabolic decompensation.

Overall, these data provide further support to the concept that T1D is not a single disease but that distinct endotypes exist. Further understanding of these endotypes is essential to inform the design of future immunotherapeutic interventions to arrest or prevent T1D.

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10.6. Progression of type 1 diabetes from latency to symptomatic disease is predicted by distinct autoimmune trajectories

Kwon BC, Anand V, Achenbach P, Dunne JL, Hagopian W, Hu J, Koski E, Lernmark Å, Lundgren M, Ng K, Toppari J, Veijola R, Frohnert BI

T1DI Study Group.

Nat Commun. 2022 Mar 21;13(1):1514.

https://pubmed.ncbi.nlm.nih.gov/35314671/

Brief Summary: This study of 5 birth cohorts of individuals at high risk for type 1 diabetes (T1D) used machine learning methods to explore trajectories from autoantibodies appearance to T1D progression. They identified 11 distinct latent health states and individuals progressed according to one of three distinct trajectories (TR1, TR2 and TR3), with an associated 5-year cumulative diabetes-free survival of 40%, 62%, and 88%, respectively.

T1D autoantibodies are markers of the autoimmune process and there is strong evidence that their presence predicts the risk of developing T1D (1). However, their temporal appearance and progression shows heterogonous patterns, which could further support and refine risk stratification. To better understand these patterns, the researcher investigated the presence or absence of three islet autoantibodies (GADA, IAA, and IA-2A), prior to the onset of clinical diabetes, in a large cohort of over 24,000 participants at risk of T1D recruited in 5 prospective studies (DAISY, DiPiS, DIPP, DEW-IT, BABYDIAB).

Using machine learning methods, a model containing 11 latent states was discovered that best fits the data and was subsequently applied to all autoantibody positive participants. Over 15 years of follow up, 643 participants developed T1D and the analysis identified three trajectories, TR1, TR2, and TR3, each characterized by a distinct sequence of 11 identified latent states. The three trajectories were associated with a different risk of developing T1D. Participants in TR1 progressed faster to diabetes than those in TR2, who progressed faster than those in TR3. Age, sex, and HLA-DR status further refined the progression rates within trajectories, improving risk stratification.

The defined trajectories and associated visual representation of the latent status within each of them could support screening strategies and be implemented in clinical practice. Clinicians can use autoantibodies patterns and age to estimate the trajectory of their patient and therefore their risk for developing T1D.

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10.7. Circulating C-peptide levels in living children and young people and pancreatic beta cell loss in pancreas donors across type 1 diabetes disease duration

Carr ALJ, Inshaw JRJ, Flaxman CS, Leete P, Wyatt RC, Russell LA, Palmer M, Prasolov D, Worthington T, Hull B, Wicker LS, Dunger DB, Oram RA, Morgan NG, Todd JA, Richardson SJ, Besser REJ

Diabetes. 2022;71:1591-1596.

https://pubmed.ncbi.nlm.nih.gov/35499624/

Brief Summary: This cross-sectional study compared trends in plasma C-peptide decline in 4,076 young people with type 1 diabetes (T1D), with trends in beta-cell loss in 235 pancreas donors. As expected, C-peptide declined over time, and this was particularly marked in children with T1D younger than 7 years. Of interest, plasma C-peptide profiles were mirrored by trends of loss of islets containing beta-cells within pancreas sections.

C-peptide is a useful indicator of beta-cell function, whose levels can be detected even after many years after the onset of T1D (1). This residual beta cell function has been associated with better glycemic control and reduced risk of severe hypoglycemia, as well as reduced long-term complications (2). Although several studies have shown a decline in C-peptide over time, measuring residual C-peptide alone cannot distinguish between loss of beta cell mass and reduced functionality. Clarifying this is important in view of the development of interventions to preserve residual beta cell function.

This is the first study comparing pancreatic histology with patterns of C-peptide loss according to age at diagnosis (<7, 7–12, ≥ 13 years) and duration (<1, 1–5, 5–10, ≥ 10 years) in young people with T1D. This study adds to the existing evidence by showing that progressive loss of insulin-containing beta cells is the main contributory factor to the decline in endogenous insulin secretion, as measured by C-peptide. This supports the utility of C-peptide as marker of residual beta cell mass for clinical indications and in the context of clinical studies.

The study also highlights that a younger age at T1D diagnosis is associated with a steeper decline in circulating C-peptide and beta cell mass. An additional interesting finding was that up to 5% of young children can retain C-peptide production 10 years after diabetes onset, reiterating the concept of heterogeneity not only across age groups but also within each age group. This heterogeneity is a key factor to consider when designing clinical trials aiming at preserving residual beta cell function and still needs further clarification of the underlying mechanisms.

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10.8. Insulin is expressed by enteroendocrine cells during human fetal development

Egozi A, Llivichuzhca-Loja D, McCourt BT, Bahar Halpern K, Farack L, An X, Wang F, Chen K, Konnikova L, Itzkovitz S

Nat Med. 2021 Dec;27(12):2104-2107.
https://pubmed.ncbi.nlm.nih.gov/34887578/

Brief Summary: This study used single-cell transcriptomic analyses to generate a cell atlas of the human fetal and neonatal small intestine. Notably, the authors identified a subset of fetal enteroendocrine K/L cells (named FIKL) that express high levels of insulin and other beta cell genes.

This study explored whether insulin is expressed in the enteroendocrine cells of the human fetus. There is a strong rationale for focusing on the fetal gut due to the shared developmental origins between the pancreas and small intestine as well as common transcriptional programmes and stimulus-secretion mechanisms between the intestinal enteroendocrine and pancreatic islet cells (1). Comparative analysis between fetal and neonatal cells led to the remarkable finding that, in the fetal small intestine enteroendocrine K/L cells expressed high levels of the insulin gene, along with other typical beta cell genes. Although the newborn's small intestines also contained K/L cells, these did not express insulin.

While all cells contain the insulin gene, only pancreatic beta cells can secrete insulin. Hence, this finding of additional insulin-expressing fetal cells is notable. At present, the mechanisms that turn off FIKL cell's insulin-making program at birth remain unknown, as well as whether this can be turned back on. The potential paracrine or systemic role of the FIKL cells also remains to be clarified. The finding that the FIKL cells were not detected in all analysed samples raises the question as to whether their expression might be driven by specific environmental conditions, such maternal gestational diabetes and exposure to high glucose levels during fetal life.

Overall, these findings create new hopes for the identification of extra-pancreatic sources of beta cells, which is a promising avenue for the treatment of diabetes.

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Advances in Clinical Practice

10.9. Comparison of insulin dose adjustments made by artificial intelligence based decision support system and by physicians in people with type 1 diabetes using multiple daily injections therapy

Nimri R, Tirosh A, Muller I, Shtrit Y, Kraljević I, Alonso MM, Milicic T, Saboo B, Deeb A, Christoforidis A, den Brinker M, Bozzetto L, Bolla AM, Krcma M, Rabini RA, Tabba S, Vazeou-Gerasimidi A, Maltoni G, Giani E, Dotan I, Liberty IF, Toledano Y, Kordonouri O, Bratina N, Dovc K, Biester T, Atlas E, Phillip M

Diabetes Technol Ther. 2022;24:564-572.
https://pubmed.ncbi.nlm.nih.gov/35325567/

Brief Summary: This physician survey-based study compared insulin dose recommendations between an artificial intelligence-based decision support system (ED-DSS) and 20 experienced physicians from 11 countries. Using data from 17 individuals with type 1 diabetes (T1D) treated with multiple daily insulin injections (MDI), the proportion of agreement and disagreement for insulin dose adjustment observed between the ED-DSS and physicians was statistically non-inferior to that among physicians.

This study explored one specific area of T1D management, insulin dose adjustments, in individuals using MDI. This typically requires healthcare professionals to provide constant advice and support to the patients and families during clinic appointments and in between visits.

Artificial intelligence is becoming an attractive tool in several medical fields, and previous studies provided interesting data for its role for T1D management, primarily for dose adjustments in patients using insulin pumps (1,2). The Endo.Digital (ED-DDS), used in this study, is a software designed to provide a comprehensive analysis of individual glucose control and advice on insulin treatment plan (1). Of interest, recommendations for insulin dose adjustments made by this automated system did not differ significantly from those given by expert physicians regarding the direction of change, and they were even more cautious for the magnitude of change in insulin dose adjustments.

Although based on data reviewed by a limited number of clinicians from different centres, these results support the use of an automated system as a tool which could be implemented in clinical practice to assist health care professional in managing people with T1D using MDI therapy, especially when and where accessibility to expert clinics is limited.

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10.10. Telemedicine and COVID-19 pandemic: the perfect storm to mark a change in diabetes care results from a world-wide cross-sectional web-based survey

Giani E, Dovc K, Dos Santos TJ, Chobot A, Braune K, Cardona-Hernandez R, De Beaufort C, Scaramuzza A
ISPAD Jenious Group.
Pediatr Diabetes. 2021;22:1115-1119.
https://pubmed.ncbi.nlm.nih.gov/34741569/

Brief Summary: This cross-sectional survey describes healthcare professionals' (HCPs) experiences of telemedicine use in diabetes centers across the world, along with the adaptations and challenges associated with its implementation.

This is one out of several publications reporting data on the emerging role of telemedicine in diabetes as well as other specialties during the COVID-19 pandemic. Diabetes is well-suited for telemedicine given that the individuaĺs treatment data can be upload into specific platforms and shared electronically and discussed between HCPs and patients/families during video-consultations (1). The COVID-19 pandemic accelerated an ongoing process of digital transformation in healthcare and introduced new challenges and opportunities to patients/families, and HCPs (2).

This article is unique in providing an overview of the experience with the use of telemedicine and related positive aspects as well as drawbacks, worldwide through a collection of data from 209 HCPs from 33 countries, all members of the International Society for Pediatric and Adolescent Diabetes (ISPAD). The results of the survey convincingly show that the proportion of young people with diabetes receiving telemedicine visits increased worldwide from $<10\%$ (pre-pandemic) to $>50\%$ (during the pandemic).

Although HCPs' satisfaction with the use of telemedicine was high (over 80%), they highlighted the need for several changes, such as an increase in specific privacy requirements for remote visits, data protection policies and reimbursement for remote care. In addition, challenges with the use of different platforms and the need of extra-time for video-consultations remain to be solved.

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Complications and Comorbidities

10.11. Thirty-Year time trends in diabetic retinopathy and macular edema in youth with type 1 diabetes

Allen DW, Liew G, Cho YH, Pryke A, Cusumano J, Hing S, Chan AK, Craig ME, Donaghue KC

Diabetes Care. 2022 May 20:dc211652.

https://pubmed.ncbi.nlm.nih.gov/35594057/

Brief Summary: This longitudinal study reports trends in diabetic retinopathy (DR) and macular edema (DME) across 3 decades: 1990-1999, 2000-2009, 2010-2019, in a large Australian cohort of 2404 adolescents with type 1 diabetes (T1D). The prevalence of DR decreased between 1990-1999 and 2000-2009, from 40 to 21%, and remained stable thereafter (20%), whereas the prevalence of DME remained low across the three decades 1.4, 0.5, and 0.9%.

Diabetic retinopathy is the most common eye diseases and a main cause of blindness among people with T1D, therefore contributed to the overall morbidity associated with this condition. The early identification and treatment of this complication is of paramount importance (1). Understanding the contemporary prevalence and severity of DR in the adolescent population is important to inform screening guidelines (2).

The study showed an important decrease in the prevalence of DR between 1999 and 2009 but a static trend during the last decade when, however, still one in five adolescents had DR.

The main risk factors included some known ones such as high HbA1c, longer diabetes duration, and some emerging factors such as overweight/obesity, diastolic blood pressure. Of note, the use of insulin pump therapy (CSII), which increased during the first two decades, was associated with 38% lower risk of DR than MDI, independently of HbA1c, likely reflecting more physiological insulin delivery or less glycemic variability with CSII.

This study was based on a long follow up and standardized protocols to grade DR in one of the main centres with expertise in diabetic retinopathy. However, the findings might be country specific and not generalizable. Nevertheless, these findings are important and support current recommendations to lower glycemic targets, increase CSII use, and target modifiable risk factors including blood pressure, cholesterol, and overweight/-obesity, to prevent DR and other vascular complications (2).

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10.12. Bone mineral density and type 1 diabetes in children and adolescents: a meta-analysis

Loxton P, Narayan K, Munns CF, Craig ME

Diabetes Care 2021;44:1898-1905.

https://pubmed.ncbi.nlm.nih.gov/34285100/

Brief Summary: This systematic review and meta-analysis of 46 studies and 6,468 participants (aged <20 years) provides evidence that youth with type 1 diabetes (T1D) have reduced lower body mineral density (BMD), as assessed by multiple modalities: DXA, peripheral quantitative computed tomography (pQCT), and/or quantitative ultrasound (QUS).

Assessing bone health in people with T1D is important based on adult studies suggesting an increased fracture risk compared to the general population, due to reduced BMD and poor bone quality (1). Data in children and adolescents with T1D are less clear and consequently, although bone health is considered as one of the comorbidities of T1D in international guidelines, there are no clear recommendations on screening.

This meta-analysis, although limited by heterogeneity between the included studies and the assessment of BMD by pQCT or QUS in only few studies, is the first to examine multiple modalities to assess BMD. DXA is the classical method used for BMD although it can be limited by its dependency on body size and lack of reporting z-scores in several studies. QUS and pQCT, which are primarily used in the research settings, may be more appropriate for assessing a maturing skeleton, being less influenced by bone size and use less radiation (2).

Youth with T1D had lower total body, spine, and femoral neck BMD, as well as lower total body BMD and lumbar spine BMD z scores (by DXA) and lower phalangeal and calcaneal BMD (QUS). pQCT demonstrated a differential effect of T1D on the trabecula and cortical components of bone. The only factor associated to BMD was age. However, there was high heterogeneity across studies in reporting other variables which could have affected the results.

It is surprising that HbA1c was not associated to BMD, and this can be explained by inclusion of only cross-sectional HbA1c values, therefore limiting the assessment of longterm glycemic control. Chronic hyperglycemia was previously hypothesized to induce altered osteoblast differentiation and maturation and alteration of osteoclast activity (3). Overall, these findings suggest that routine assessment of BMD should be considered in youth with T1D, although further data are needed to support that. Similarly, there is a need to understand the mechanisms of abnormal bone development in T1D to inform recommendations for prevention.

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Pathogenesis

10.13. Human islet T cells are highly reactive to preproinsulin in type 1 diabetes

Anderson AM, Landry LG, Alkanani AA, Pyle L, Powers AC, Atkinson MA, Mathews CE, Roep BO, Michels AW, Nakayama M
Proc Natl Acad Sci U S A. 2021 Oct 12;118(41):e2107208118.
https://pubmed.ncbi.nlm.nih.gov/34611019/

Brief Summary: This in vitro study explored the reactivity to preproinsulin of CD8 T cells obtained from pancreatic islets of individuals with and without type 1 diabetes (T1D). The main finding was that CD8 T cells from T1D donors were highly reactive to peptides throughout the entire preproinsulin protein.

This study focused on the cytotoxic CD8 T cells, which play a key role in the pathogenesis of T1D, where they are implicated in beta-cells destruction through self-antigen presentation by HLA class I molecules (1). The first key finding was the huge difference in the number of CD8 T cells between T1D donors and controls, and that CD8 T cells highly specific to preproinsulin are enriched within the islets of a subset of T1D organ donors. Of interest, several different HLA class I molecules emerged to have a role in presenting epitopes throughout the preproinsulin protein to the CD8 T cells. The results are in line with a previous study showing that preproinsulin-specific T cells are present in the pancreatic exocrine compartment of organ donors without diabetes, and during the course of T1D development, they accumulate into insulin-containing islets (2).

Of note, there was variability among T1D organ donors in the frequency of preproinsulin reactive CD8 T cells in the islets. This heterogeneity was associated with certain HLA alleles. The study also showed that multiple HLA class I molecules across HLA-A, -B, and -C can present epitopes of preproinsulin to activate islet derived CD8 T cells.

Many clinical trials have tested proinsulin- or insulin-based therapies to prevent T1D onset and induce tolerance (3). These approaches have proved safe but showed heterogeneous efficacy with subsets of responders. The present study provides important information for the design of future immunotherapy interventions that should consider the use of preproinsulin rather than just insulin or proinsulin and consider participants selection based on HLA typing.

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10.14. An autoimmune stem-like CD8 T cell population drives type 1 diabetes

Gearty SV, Dündar F, Zumbo P, Espinosa-Carrasco G, Shakiba M, Sanchez-Rivera FJ, Socci ND, Trivedi P, Lowe SW, Lauer P, Mohibullah N, Viale A, DiLorenzo TP, Betel D, Schietinger A

Nature 2022;602(7895):156-161.

https://pubmed.ncbi.nlm.nih.gov/34847567/

Brief Summary: This study used a well-characterized mouse model of type 1 diabetes (T1D), the non-obese diabetic mouse (NOD), to examine the fate of CD8 T cells over the 5–30 week course of the disease. They found a stem-like autoimmune progenitor population in the pancreatic draining lymph nodes, which can self-renew and generate short-lived autoimmune mediators that migrate to the pancreas, where they differentiate further and destroy β-cells.

This study provides new insights into the steps leading to T1D in a mouse model, by focusing on CD8 T cells that specifically recognize the β-cell 'islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP)'. Two different populations of IGRP-specific CD8 T cells were identified: one in the pancreatic lymph nodes, showing a high expression of the transcription-factor protein TCF1; and the other in the pancreas, which showed a more differentiated phenotype, including low expression of TCF1. Of note, TCF1 promotes self-renewal and is essential for the maintenance of memory CD8 T cells.

The CD8 T cells in the pancreas represent short-lived cells which destroy β-cells, whereas the lymph nodes cells represent stem-like autoimmune progenitors, with self-renewal properties (due to high expression of TCF1), and able to sustain the pool of cells entering the pancreas. These data show that the autoimmune progenitor cells act as a self-sustaining reservoir in the pancreatic lymph node and are the source of the disease-causing autoimmune mediators. Of note, in contrast to CD8 T cells found in the context of chronic infections or cancer, pancreatic autoimmune T cells do not display a transcriptional or phenotypic TOX-driven exhaustion program (which under conditions of chronic antigen exposure leads to T-cell exhaustion), and thus they retain effector function.

These findings suggest that strategies aimed at targeting the stem-like autoimmune progenitor pool, which represents the 'seeds' for T1D, could emerge as new effective immunotherapy interventions.

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10.15. Single-cell multi-omics analysis of human pancreatic islets reveals novel cellular states in type 1 diabetes

Fasolino M, Schwartz GW, Patil AR, Mongia A, Golson ML, Wang YJ, Morgan A, Liu C, Schug J, Liu J, Wu M, Traum D, Kondo A, May CL, Goldman N, Wang W, Feldman M, Moore JH, Japp AS, Betts MR, HPAP Consortium, Faryabi RB, Naji A, Kaestner KH, Vahedi G

Nat Metab. 2022 Feb;4(2):284-299.

https://pubmed.ncbi.nlm.nih.gov/35228745/

Brief Summary: This study used three high-throughput single-cell technologies to generate a pancreatic islet cell atlas from 24 organ donors with type 1 diabetes (T1D), autoantibody positive and healthy donors. The most remarkable finding was that a subset of exocrine ductal cells appears to acquire a signature of tolerogenic dendritic cells in an attempt at immune suppression in donors with T1D.

The pathogenesis of T1D is complex and still an area of intense research. Exploring the events leading to the development of autoimmunity and T1D is limited by the inability to safely perform pancreatic biopsies in living donors. In addition, most studies are performed in people with clinically manifested disease, in whom a substantial amount of beta cell mass has been lost (1).

Fasolino et al. provide insights into the role of pancreatic ductal cells using a pancreatic islet single-cell atlas generated by the Human Pancreas Analysis Program and studying donors with T1D along with autoantibody-positive and healthy donors. The main finding was that ductal cells of the exocrine compartment from T1D donors expressed high levels of MHC class II and interferon pathways, were surrounded by CD4+ T cells and dendritic cells and were transcriptionally like tolerogenic dendritic cells. This may indicate that ductal cells show a tolerogenic response to chronic T cell infiltration in the pancreas and appear to be an unsuccessful effort to stop the T cell response that contributes to beta cell damage.

Of interest, the study also provided some remarkable information on what happens in the pancreas of individuals at risk of T1D, and specifically those who are GAD positive. These donors showed similar transcriptional changes to T1D donors in various endocrine and exocrine cells. Although it remains to be determined whether these transcriptional changes contribute to or are a consequence of disease pathogenesis, these findings are a step forwards in the understanding of early pancreatic changes occurring in T1D.

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Genetics

10.16. Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes

Robertson CC, Inshaw JRJ, Onengut-Gumuscu S, Chen WM, Santa Cruz DF, Yang H, Cutler AJ, Crouch DJM, Farber E, Bridges SL Jr, Edberg JC, Kimberly RP, Buckner JH, Deloukas P, Divers J, Dabelea D, Lawrence JM, Marcovina S, Shah AS, Greenbaum CJ, Atkinson MA, Gregersen PK, Oksenberg JR, Pociot F, Rewers MJ, Steck AK, Dunger DB; Type 1 Diabetes Genetics Consortium, Wicker LS, Concannon P, Todd JA, Rich SS

Nat Genet. 2021 Jul;53(7):962-971.

https://pubmed.ncbi.nlm.nih.gov/34127860/

Brief Summary: The authors report the largest and most diverse genome-wide association study (GWAS) of type 1 diabetes (T1D) to date, including 61,427 participants from different ancestries. It identified 78 significant genomic regions associated with T1D, of which 36 were new. The integration of genetic evidence, functional genomic maps, and immune protein–protein interactions led to the characterization of 12 genes implicated in T1D that are already targets in clinical trials for autoimmune diseases.

In recent years, large GWASs have shed light on genetic contributors to T1D. However, most have included only people of European ancestry, whereas other ancestries have been underrepresented (1). The current GWAS study

included a diverse population and provides additional remarkable information on the complex genetic landscape of T1D. The study also addressed the mechanisms underlying the genetic T1D associations, which up to now were largely unknown. They identified up to 78 genetic regions associated with T1D, with 36 being new, and some also associated with other autoimmune conditions. The study highlighted that these regions are enriched in immune-cell accessible chromatin, particularly CD4+ effector T cells. The analysis of $CD4^+$ T cells from 115 people (58 with T1D and 57 controls) identified five regions where T1D risk variants co-localize with chromatin accessibility quantitative trait loci. Of note, rs72928038 in *BACH2* was identified as a candidate causal T1D variant. *BACH2* haploinsufficiency has been associated with congenital autoimmunity and immunodeficiency, supporting its role in the immune system. Further analysis of the genetic data led to the identification of 12 genes, which have already been targeted in trials for other autoimmune disease and which could represent targets for future drugs to arrest or prevent T1D.

Overall, this large study sheds new light on the complex T1D genetic background and unveils several new potential drug targets.

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New Treatments

10.17. Efficacy of glucagon-like peptide-1 and estrogen dual agonist in pancreatic islets protection and preclinical models of insulin-deficient diabetes

Fuselier T, Mota de Sa P, Qadir MMF, Xu B, Allard C, Meyers MM, Tiano JP, Yang BS, Gelfanov V, Lindsey SH, Dimarchi RD, Mauvais-Jarvis F

Cell Rep Med 2022;3:100598.

https://pubmed.ncbi.nlm.nih.gov/35492248/

Brief Summary: This study used a combination of mice models and cultured human islets to show that a glucagon-like peptide-1 (GLP-1) and estrogen (E2) dual agonist (GLP1-E2) provides superior protection from insulin-deficient diabetes compared to GLP-1 and E2 monoagonists.

Pancreatic islet protection is a major therapeutic goal in diabetes research. Estrogens are known to have antidiabetic properties, and previous studies showed their ability to promote beta cell survival and function both in animal models and cultured human islets (1). However, the use of estrogens as a potential diabetes preventive therapy is limited by their adverse reproductive effects. The dual agonist GLP-1-E2, used in this study, represents a potential way to selectively direct estrogens to pancreatic beta cells, which express both GLP-1 (GLP-1R) and estrogen receptors (ER-α) (2). Through a series of elegant studies, clear evidence emerged that the GLP1-E2 enhances GLP-1-mediated protection of insulin deficient diabetes in mice and that it requires the presence of both GLP-1R and estrogen receptors to exert its functions.

The dual agonist GLP1-E2 activates ER-α following GLP-1R internalization and lysosomal acidification. GLP1-E2 amplifies antiapoptotic pathways activated by GLP-1 in human beta cells. The findings also suggest that the GLP1-E2 antidiabetic actions involve GLP-1R-expressing cells outside the beta cells. Of note, GLP1-E2 did not have any feminizing effects in treated male mice, nor any effect on blood pressure or heart rate.

This study opens new avenues for research to optimize GLP1–E2 agonists, which in future could be a therapeutic option to protect functional β-cell mass in early stages of T1D.

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11. Obesity and Weight Regulation

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Preface

As in previous years, in this year's chapter we can present only 1% of the acquired publications (1326) according to our search criteria in PubMed in the Yearbook 2022. 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. The papers included in this chapter of the Yearbook are organized under these subheadings:

- Genetic obesity: findings in clinical cohorts – how to interpret results?
- New findings in adipose tissue biology
- New predictors of obesity development
- Weight regulation and endocrine circuits (including interventions).

Genetic Obesity: Findings in Clinical Cohorts – How to Interpret Results?

11.1. Testing for rare genetic causes of obesity: findings and experiences from a pediatric weight management program

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International Journal of Obesity 2022 May 13:1–9.

Doi: 10.1038/s41366-022-01139-7.

https://pubmed.ncbi.nlm.nih.gov/35562395/

Brief Summary: This exploratory study presents the findings and experiences from n = 117 children with obesity (BMI $\geq 97^{th}$ percentile for age and sex) participating in a pediatric weight management program over an 18-month period in which targeted DNA sequencing was performed for 40 genes known to cause rare genetic disorders of obesity. No homozygous or compound heterozygous variants were identified, but 72 patients (61.5%) had at least one heterozygous variant among the analyzed genes, of which 22 patients (18.8%) had 2–4 variants.

Testing for rare genetic diseases of obesity is recommended in children with early-onset obesity (%$BMI_{P95} \geq 120\%$ before 5 years of age) that have clinical features of genetic obesity syndromes (including hyperphagia) and/or a family history of severe obesity [1]. Most variants identified in this cohort were rare ($< 0.05\%$ minor allele frequency in gnomAD) [2] and of uncertain significance according to the ACMG guidelines [3] (4 patients had variants classified as "likely pathogenic/pathogenic" in autosomal recessive inherited genes, 8 patients a PCSK1 variant classified as "risk" factor, 1 patient a MC4R variant classified as "likely pathogenic" in addition to the PCSK1 "risk" variant). In contrast, previous studies on genetic obesity disorders reported an underlying causative genetic defect in 2–13% of pediatric patients with severe obesity, excluding variants of uncertain significance [4,5].

This study clearly shows the current challenge of dealing with findings of genetic tests that identify variants of uncertain significance and highlights the need to develop clinical tools to increase provider confidence in offering genetic testing and communicating genetic results with families. Further research in larger cohorts, which includes

detailed phenotypic characterization of the patients, segregation and functional analysis is needed to understand how variants of uncertain significance or multiple variants influence obesity and hyperphagia.

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11.2. Rare variant analysis of obesity-associated genes in young adults with severe obesity from a consanguineous population of Pakistan

Saeed S, Janjua QM, Haseeb A, Khanam R, Durand E, Vaillant E, Ning L, Badreddine A, Berberian L, Boissel M, Amanzougarene S, Canouil M, Derhourhi M, Bonnefond A, Arslan M, Froguel P
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Diabetes 2022;71:694–705
https://doi.org/10.2337/db21-0373

Brief Summary: In this observational cross-sectional study in 128 randomly selected young obese adults (BMI 37.2 ± 0.3 kg/m^2; age 18.4 ± 0.3 years) from the Severe Obesity in Pakistani Population (SOPP), screening by whole-exome analysis found 3% had (likely) pathogenic variants in the leptin-melanocortin pathway and a further 4 had a CNV and 11 had rare, homozygous non-synonymous variants in 15 different genes of interest.

Pakistan is known both for a high degree of consanguineous marriages [1] and high prevalence of obesity *[2]*. Thus, this country presents a unique opportunity to study autosomal recessive forms of obesity. Accordingly these authors had previously identified in their SOPP Children cohort loss of function in *ADCY3* as a new form of monogenic obesity [3]. Here, they present further analysis of this interesting cohort with advanced molecular-genetic methods. The data are of high importance for the research community especially in terms of the need of a critical assessment of new genes potentially involved in weight regulation

Nevertheless, the identified variants display all the difficulties of identifying new pathogenic genes: For only two of the genes, mice knock-out models with increased obesity exist (*ASNSD1* and *IFI16*) [4,5]. Though in the other genes, polymorphisms have been associated with obesity in GWAS, for only one gene (*ABTB1*), heterozygous LoF variants were associated with an increased risk for obesity in the UK Biobank population. And only 2 variants (*B4GALNT3* and *DNAJC27*) were predicted by both SIFT analysis and Mutation Taster to be deleterious/disease causing. Thus, it remains to be proven if any of the here identified genes truly are the cause of obesity in these patients.

A further surprising finding is the low rate of only 3% of (likely) pathogenic variants in the leptin-melanocortin pathway in this cohort – in contrast to 30%–40% in the SOPP – Children cohort [6,7]. A lower yield of monogenic obesity in adult versus childhood cohorts has already been described by others [8]. One reason might be the additional selection criteria in the childhood cohort (parents had to be non-obese and consanguineous) [7]. The authors also argue that a high early mortality rate and/or severe disabilities of affected children prevent them to enter mainstream young adult populations.

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New Findings in Adipose Tissue Biology

11.3. Isthmin-1 is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis

Jiang Z, Zhao M, Voilquin L, Jung Y, Aikio MA, Sahai T, Dou FY, Roche AM, Carcamo-Orive I, Knowles JW, Wabitsch M, Appel EA, Maikawa CL, Camporez JP, Shulman GI, Tsai L, Rosen ED, Gardner CD, Spiegelman BM, Svensson KJ
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Cell Metab 2021; 33(9): 1836–1852.e11
http://www.ncbi.nlm.nih.gov/pubmed/34348115

Brief Summary: This rodent study identified a novel adipokine in mice which triggers a signaling cascade similar to that of insulin. By acting via an unknown tyrosine kinase, isthmin-1 (ISM1) ameliorates metabolic disturbances associated with type 2 diabetes mellitus, including hyperglycemia and liver steatosis. Interestingly, *Ism1* had been discovered some years ago as a gene expressed in the Xenopus midbrain-hindbrain organizer called *isthmus* and it had been suggested to be important for early brain development [1–3].

Glucose homeostasis is regulated by the interplay of glucose uptake in insulin-sensitive tissues (adipose, muscle, heart) on the one hand, and gluconeogenesis by liver, kidneys, and gut on the other hand [4]. Upon hyperinsulinemic conditions, insulin promotes lipogenesis in the liver contributing to fatty liver disease, which is a side-effect of insulin therapy. According to the data of this paper, ISM1 has a dual role in both activating the PI3K/Akt cascade in adipose tissue, skeletal muscle and liver and inhibiting lipogenesis in the liver at the same time. These properties of ISM1 argue for its potential as a molecule useful in diabetes therapy. Indeed, upon long-term treatment in mice (21 days), ISM1 improved glucose tolerance and insulin sensitivity. In another model of non-alcoholic fatty liver disease, ISM1 treatment was effective on reducing liver lipid accumulation. Further, overexpression of ISM1 prevented DIO-induced insulin resistance and hepatic steatosis.

Some questions remain unanswered so far. To assess its suitability as an anti-diabetes drug, the identification of the ISM1 receptor is crucial. Further, regulation of ISM1 serum levels should be determined under pathological conditions in order to understand if ISM1 treatment would be effective in obese individuals.

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11.4. Expression of the adipocyte progenitor markers MSCA1 and CD36 is associated with adipose tissue function in children

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JCEM 2022; 107(2): e836–e851
https://pubmed.ncbi.nlm.nih.gov/34448000

Brief Summary: This study investigated the association of the novel adipose-derived stem cell (ASC) markers Mesenchymal Stem cell Antigen 1 (MSCA1) and CD36 with adipose tissue function in samples collected from children undergoing elective surgery in the Leipzig AT Childhood Cohort.

Previous work demonstrate that different subpopulation of adipocyte progenitor cells exist which differ in terms of proliferation and differentiation [1,2]. These cells are of particular interest in obesity research as they play a crucial role in adipose tissue development and the pathogenesis of obesity. However, the ASC population is not well defined in humans, and more research is needed to better define the different ASC subsets and the function of ASC marker genes. The surface markers CD36 and MSCA1 have been recently connected to adipogenic differentiation and adipocyte metabolism [3,4], suggesting that these genes might play a role during obesity-related AT accumulation.

Here, the authors found that CD36 expression was decreased in children with overweight and obesity and was positively associated with the differentiation capacity of ASCs *in vitro*. Expression of MSCA1 was positively correlated with traits of AT dysfunction (adipocyte hypertrophy and C-reactive protein) and was further associated with increased mitochondrial respiration *in vitro*.

Expansion of adipose tissue is either regulated by hypertrophy (e.g. increase in adipocyte volume) or hyperplasia (e.g. increase in cell number), the latter dependent on adipogenic differentiation from progenitor cells within the adipose tissue. It is thus crucial to identify biomarkers that define adipocyte progenitor subpopulations associated with early obesity. In previous studies, the role of CD36 and MSCA1 has been evaluated in adult patients with morbid obesity [3,5], but their role in early obesity progression has not been addressed so far. Thus, the strength of this study is that it was performed in healthy children, allowing a better insight into early processes involved in obesity development. Future studies may address the molecular mechanisms that link these progenitor makers with early obesity progression.

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11.5. Insulin directly regulates the circadian clock in adipose tissue

Tuvia N, Pivovarova-Ramich O, Murahovschi V, Lück S, Grudziecki A, Ost AC, Kruse M, Nikiforova VJ, Osterhoff M, Gottmann P, Gögebakan Ö, Sticht C, Gretz N, Schupp M, Schürmann A, Rudovich N, Pfeiffer AFH, Kramer A
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Diabetes 2021;70:1985–1999
http://www.ncbi.nlm.nih.gov/pubmed/34226282

Brief Summary: This study analyzed gene expression in subcutaneous adipose tissue samples of adults with obesity collected before and after the hyperinsulinemic–euglycemic clamp or control saline infusion. They demonstrate that insulin regulates clock genes in both murine and human adipocytes and adipose-derived stem cells (ASCs).

Circadian clocks are a system of self-sustained oscillators that regulate various physiological processes through the generation of approximately 24h circadian rhythms in gene expression, which are translated into rhythms in metabolism and behavior. Clock genes are set by external cues (so-called “zeitgeber”) to synchronize

endogenous rhythms with environmental cycles. An important "zeitgeber" of peripheral tissues is food intake [1], and previous publications suggest that this might be mediated by insulin action [2,3].

Using reporter assays, the authors could show that insulin directly shifts the time-dependent expression of period circadian regulator 2 *PER2*. They further identified a region in the *PER2* gene harboring potential binding sites for the transcriptional regulators NFY and SP1, which are downstream of the insulin signaling cascade. This suggests that insulin directly influences the circadian clock in adipose tissue by transcriptional regulation of clock genes.

The paper contributes to the long debate on the existence of a food-driven oscillator [4], which links feeding with peripheral circadian clock setting. This implicates that every time we eat, we reset our internal clock to synchronize with adipose tissue metabolism. This might also explain why rapid changes in eating behavior (e.g. due to jet lag, shift work or nighttime feeding) disturbs metabolic homeostasis. It would be interesting to further identify the downstream signals that mediate the effect of insulin on clock genes in the adipose tissue.

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11.6. Obesity and hyperinsulinemia drive adipocytes to activate a cell cycle program and senesce

Li Q, Hagberg CE, Cascales HS, Lang S, Hyvönen MT, Salehzadeh F, Chen P, Alexandersson I, Terezaki E, Harms MJ, Kutschke M, Arifen N, Krämer N, Aouadi M, Knibbe C, Boucher J, Thorell A, Spalding KL

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Nat Med 2021; 27(11): 1941–1953

doi: 10.1038/s41591-021-01501-8

Brief Summary: This study analysed adipocytes from obese and hyperinsulinemic adults. They demonstrate that obesity is associated with the induction of a cell cycle program in mature adipocytes, leading to so-called endoreplication, as a strategy to remain functionally active. Under hyperinsulinemic conditions, this program fails and leads to cell-cycle exit and premature senescence.

Adipose tissue expands either by generating new adipocytes from progenitor cells (hyperplasia), or by enlargement of preexisting adipocytes (hypertrophy). While hypertrophic adipose tissue is generally considered a metabolically beneficial, hyperplastic adipose tissue is associated with an increased risk to develop metabolic disease [1]. Here, the authors used RNA sequencing of mature adipocytes from obese and hyperinsulinemic adults to demonstrate that mature adipocytes, although considered post-mitotic, can re-enter cell cycle, and that this cell cycle progression correlated with obesity and hyperinsulinemia. However, the cells do not enter mitosis, but increase their DNA content, suggesting that these adipocytes undergo endoreplication, i.e. replicate their genome in the absence of cell division. Endoreplication in other cell types has been described as a program to enhance cellular DNA content to respond to an increased demand of cell size, protein biosynthesis, or nutrient storage. The authors therefore suggest that this might be a response mechanism in adipocytes to remain functionally active. Upon prolonged hyperinsulinemia, however, adipocytes undergo cellular senescence, which might contribute to adipose tissue inflammation.

This study is of general interest since it shows for the first time that terminally differentiated adipocytes can re-enter the cell cycle in order to cope with metabolic stress. It would be interesting to investigate whether blocking of cellular senescence or elimination of those senescent adipocytes would lead to an improved metabolic outcome in obesity.

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11.7. Leptin resistance before and after obesity: evidence that tissue glucose uptake underlies adipocyte enlargement and liver steatosis/steatohepatitis in Zucker rats from early-life stages

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Int J Obes (Lond) 2022, 46(1):50–58.
DOI: 10.1038/s41366-021-00941-z

Brief Summary: This experimental study was conducted with fa/fa rats that have a homozygous defect in the leptin receptor and therefore severe leptin resistance. The animals develop extreme obesity, diabetes and metabolic syndrome. The study of young, still normal-weight animals showed that the initial hyperglycemia due to hepatic glucose overproduction is a first metabolic feature of early, severe leptin resistance. Glucose intolerance seems to be causal for the development of insulin resistance and obesity, which appear later.

Leptin is a key regulator of appetite and weight gain but most people with obesity show leptin resistance. This work addresses the fundamental question of the sequence of events in obesity development. Does the lack of action of leptin lead to the initiation of obesity and the metabolic consequences or is obesity the cause of leptin resistance? The answer is ultimately fundamental to define early intervention options and time windows for prevention and treatment.

The results show that hyperglycemia is one of the first events of early manifestation of leptin resistance. Consistent with this observation is the finding that the molecular changes leading to the expansion of adipose tissue in fa/fa rats (e.g., upregulation of glucose-6-phosphate dehydrogenase, acetyl-coenzyme A carboxylase, fatty acid synthetase) are reversible through transplantation of fat tissue from healthy rats [1]. Therefore, the molecular dysfunctions in adipose tissue are secondary rather than primary and possibly triggered by circulating rather than intrinsic factors. Increased glucose uptake by the liver plays a role in these early stages and subsequently leads to the development of NASH. Increased hepatic glucose uptake and in NASH could thus be biomarkers or diagnostic markers for leptin resistance.

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New Predictors of Obesity Development

11.8. Why do humans undergo an adiposity rebound? Exploring links with the energetic costs of brain development in childhood using MRI-based 4D measures of total cerebral blood flow

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doi: 10.1038/s41366-022-01065-8. Epub 2022 Feb 8.
https://pubmed.ncbi.nlm.nih.gov/35136192/

Brief Summary: This cross-sectional study measured total cerebral blood flow (TCBF) by MRI in 82 healthy individuals (ages 0–60 years) to explore the association between TCBF and BMI over life. Of particular interest was the question whether the BMI nadir (adiposity rebound, AR) and TCBF are inversely related.

Adiposity rebound (AR) in early childhood is a risk factor for obesity in adolescence and adulthood [1]. Aronoff *et al* propose that there is a link between AR and the energetic demand of brain development. It is assumed that AR results from changes in body composition during infancy and childhood which are determined by a shift in

energy balance. Shifts in energy balance during childhood are related to changes in level of physical activity, but also age-related changes in brain energy requirements have been shown [2,3]. A measure of brain energy requirement is the TCBF, which is closely coupled to local glucose metabolism [4,5]. Aronoff *et al* observed that TCBF and BMI were inversely correlated over the life course. In the subgroup of pre-pubertal children, the peak for the TCBF at 5.6 years was near the BMI nadir at 4.9 years of age. This observation is important, because it helps for a deeper understanding of life-course influences on body composition trajectories and related disease risk. This observation also highlights the brain as a potential pathway linking early life experiences with obesity risk. The authors hypothesize that early adversity and stressors could alter body composition trajectories by accelerating brain maturation, possible leading to an earlier peak in brain metabolism and resulting in an earlier AR and greater adiposity.

The strength of the present work is certainly the measurement of TCBF by MRI in a cohort including infants, children, adolescents and adults. The cross-sectional design is a limitation. To confirm the hypothesis that the AR is linked developmentally to the high energetic requirements of brain development at this age, longitudinal BMI and TCBF measures from birth onwards are necessary.

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11.9. Fat mass and fat-free mass track from infancy to childhood: New insights in body composition programming in early life

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Obesity (Silver Spring). 2021 Nov;29(11):1899–1906.

Doi: 10.1002/oby.23271. Epub 2021 Sep 21.

https://pubmed.ncbi.nlm.nih.gov/34549538/

Brief Summary: This longitudinal study assessed body composition in a cohort of children at ages 1, 3, and 6 months and 4 years ($n = 224$, Boys: $n = 120$). Being in the "high group" of FM% at 3 or 6 months of age was associated with a higher risk of being in the "high FM% group" at 4 years of age. The authors concluded that high percent fat mass (FM%) tracked from age 3 and 6 months to 4 years of age.

The question of tracking body composition from early childhood into adulthood is of interest. The strengths of the present study are the longitudinally and detailed measurements of body composition from birth up to 4 years of age in a group of healthy, term-born children. Body composition was measured by Air displacement plethysmography (ADP) at 1, 3 and 6 months of age and by Dual X-ray Absorptiometry (DXA) at 6 months (if children's weight was already greater than 8 kg) and at 4 years of age. Markers of body composition FM%, FMI, FFMI, abdominal subcutaneous and visceral fat and BMI-SDS were grouped in "high", "moderate" or "low" (using tertiles) at each time point.

Only few studies to date have longitudinally assessed body composition in early childhood from birth onwards [1,2]. The observations from this study add to literature where tracking of body fat mass from early life through childhood has been shown [1,2]. The knowledge about tracking of body composition from infancy to childhood and adolescents is important for clinical practice, since it is known that almost 90% of children who were obese at 3 years of age were overweight or obese in adolescence [3].

One limitation is that the analysis of the data using logistic regression does not directly test tracking. Regardless of this, well-designed cohorts like this cohort are important for a deeper understanding of tracking of body composition during childhood up to adolescence and adulthood.

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11.10. Neonatal and adolescent adipocytokines as predictors of adiposity and cardiometabolic risk in adolescence

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Obesity (Silver Spring). 2021 Jun;29(6):1036–1045.

Doi: 10.1002/oby.23160.

https://pubmed.ncbi.nlm.nih.gov/34029449/

Brief Summary: This longitudinal study assessed the association between changes in leptin and adiponectin levels from birth to 12 years of age with level of adiposity and cardiometabolic risk in adolescence in a prospective birth cohort study (HOME Study, $n = 166$). They observed that compared to adolescents with decreasing leptin z-scores, adolescents with stable or increasing leptin z-scores from birth onwards had higher adiposity and worse cardiometabolic risk scores in adolescence. Adolescents with increasing or stable adiponectin-to-leptin ratio had lower adiposity and more favorable cardiometabolic risk scores than adolescents with a decreasing adiponectin-to-leptin ratio.

It has been reported that changing leptin concentrations over time are related to higher waist circumference and BMI in midchildhood [1] as well as higher metabolic risk scores in adolescents [2]. Therefore, this paper is important because it supports previous findings and highlights a subgroup of adolescents with a specific leptin course from birth onwards, characterized by a worse cardiometabolic risk score and higher adiposity in adolescence. Leptin levels at birth and at 12 years of age were moderately positively correlated, whereas adiponectin levels did not correlate between birth and adolescence. An interesting finding was that the authors identified children with increasing, decreasing or stable leptin z-score (age- and sex-standardized), or adiponectin-to-leptin ratio from birth up to 12 years of age.

The pathophysiological mechanism behind this observation is unclear. It is possible that changing leptin concentrations over time may present the development of leptin resistance, which is associated with insulin resistance, central and visceral adiposity or it represents a partial leptin deficiency that is compensated by excess leptin production during the course. Regardless of this question, studies like this help to identify subgroups that can then be characterized subsequently, and which can thereby contribute to a better understanding of the pathophysiology of development of adiposity.

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Weight Regulation and Endocrine Circuits (Including Interventions)

11.11. The pubertal growth spurt is diminished in children with severe obesity

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doi: 10.1038/s41390-020-01234-3.

Brief Summary: This observational study compared the pubertal growth spurt of children in a Spanish study group with severe early onset obesity to children in a Swedish community-based study. The authors show that childhood obesity impairs the pubertal growth spurt in a severity-related manner also in children with extreme obesity.

Since nearly 100 years we know that prepubertal children with obesity are in general taller than normal-weight children and that being overweight or obese during childhood is associated with increased height velocity [1], accompanied by advanced bone age. In 2016, these authors developed a shape-invariant growth model, QEPS, which describes the total pattern of growth in height from fetal life to adult height based on four basic growth functions and enables researchers to identify the impact of the pubertal growth spurt on total adult height [2]. Using this model, the authors had shown in a previous study in a community-based setting an inverse linear correlation between the highest childhood BMI SDS and specific pubertal height gain; the higher the BMI during childhood, the less the pubertal height gain [3]. As that study included only a limited number of children with obesity, here they expand their results to children with extreme obesity demonstrating the higher the BMI in childhood, the lower the pubertal height gain in children also when comparing moderate to extreme obesity.

Tall childhood stature relative to parents' heights is a robust marker that obesity is nutritional in origin (rather than hormonal or syndromic) – but is not associated with taller adult height.

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11.12. Lower circulating sertoli and leydig cell hormone levels during puberty in obese boys: a cross-sectional study

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J Clin Endocrinol Metab. 2022 Mar 24;107(4):e1568–e1576.
Doi: 10.1210/clinem/dgab845
https://pubmed.ncbi.nlm.nih.gov/34918072/

Brief Summary: This cross-sectional study describes how levels of Sertoli and Leydig cell hormones differ between 351 obese and overweight boys aged 5–19 years from a normative collective of 652 healthy, non-overweight, non-obese boys of similar age and Tanner stage from France.

The authors showed that from age 12–14 years or Tanner G1-2 onwards, overweight and obese (ow/ob) boys had lower levels of inhibin B, with 22% having levels $<$5th percentile. A significant difference in testosterone levels could be shown from the age of 14 or Tanner G4 onward, with 28% of the ow/ob boys having levels $<$5th percentile. There were no differences found in gonadotrophin levels. A higher estradiol level was found already before puberty from age 8 onward, showing excess aromatization of adrenal androgens. The lower inhibin B levels were highly correlated to estradiol levels and FSH, indicating a contribution of excess aromatization, as well as primary Sertoli cell impairment. The testosterone levels were inversely correlated to fat mass measured by DXA. AMH values did not differ, but there was a higher dispersion in ow/ob boys. Lower AMH levels were physiologically correlated to testosterone but also to fasting insulin, indicating an effect of insulin resistance on Sertoli cell function. The measured effects on inhibin B and testosterone levels confirmed previous data showing altered testicular growth and hormone production in obese boys [1,2]. The precise assessments of body composition and metabolism on large numbers of boys revealed potential underlying causes of these variations. Due to the cross-sectional design, it was not possible to evaluate whether these were persistent changes, but data on adult men suggest a lasting effect on sperm production [2,3].

While there are other positive nutritional drivers of sex hormone secretion [4] these data suggest a inhibitory effect of fat mass on testosterone in boys. This may explain why some boys with OW/OB present with delayed puberty [1]. Further longitudinal research will be needed to assess these questions and whether weight loss can reverse the observed alterations.

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11.13. Weight gain and obesity rates in transgender and gender-diverse adults before and during hormone therapy

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doi: 10.1038/s41366-021-00935-x. Epub 2021 Aug 16.

PMID: 34400797.

https://pubmed.ncbi.nlm.nih.gov/34400797/

Brief Summary: This retrospective chart review of 470 ethnically diverse transgender and gender diverse individuals (> = 17 years) from two medical centres in Washington, DC, studied their weight trajectories during gender affirming hormone therapy (GAHT) over up to 57 months. The authors found an increase in body weight in trans-males receiving testosterone almost immediately after GAHT initiation (+3–4%). After that, BMI remained stable for 34 months. In trans-females, BMI first remained constant but increased after 22 months.

The baseline obesity rates of trans-males were 1.5-4x higher than in European studies [1–7] but comparable to US reference populations [8] and rose during GAHT up to 52%, while at baseline and throughout treatment, fewer trans-females individuals than *cis*-gender people were obese.

While other studies had shown weight increases for trans-males receiving testosterone during the initial year [1–7], data on weight effects of GAHT for trans-females were scarce and conflicting [1–3,7]. This study showed no direct effect during the first 21 months of treatment. Previous studies had shown that the initial weight gain in trans-males is primarily caused by a gain in lean mass while fat mass reduces [9]. As this study did not assess body composition, it is uncertain how much fat mass was gained during treatment. Also, as there was no control group, it is unclear how high the contribution of GAHT compares to other factors like aging, especially since the highest weight gain was observed in the youngest age group (17–29 years old).

Compared to previous reports, this study is the first to describe the time course of body weight changes during GAHT over more than 2 years and its effects on different weight categories and ethnic groups. It shows how vital weight management and obesity treatment are for transgender people who are already at an elevated risk for cardiovascular disease.

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11.14. Energy balance in hypothalamic obesity in response to treatment with a once-weekly GLP-1 receptor agonist

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International Journal of Obesity 2022, Epub 2022 Jan 3.
Doi: 10.1038/s41366-021-01043-6.
https://pubmed.ncbi.nlm.nih.gov/34975146/

Brief Summary: This double-blind, placebo-controlled trial assessed the efficacy of 36 weeks of GLP1RA treatment with exenatide extended release once-weekly on weight loss and energy balance in 42 patients (aged 10–26) with hypothalamic obesity (HO) caused by suprasellar tumors. The results showed a decrease in food intake but also a decrease in total energy expenditure.

Glucagon-like peptide-1 receptor agonists (GLP1RA) are known to cause weight loss in adults [1] and adolescents [2] with obesity through mechanism independent of intact hypothalamic structures. In a previous study of GLP1RA, patients with HO did not achieve weight loss but showed a slower increase in BMI and body fat than placebo controls [3]. In this second analysis in patients with HO, the authors show that GLP1RA compared to placebo reduced energy intake during a buffet meal (−1800 kJ, 95% CI – 3184 to −418 kJ, $P=0.02$) but also reduced total energy expenditure (−327 kJ/day, 95% CI −699 to −42kJ/day, $P=0.04$) measured using doubly labeled water. In contrast to the continuous increase in BMI in patients with HO treated with GLP1RA, the results of energy intake and total energy expenditure are in line with the results of previous studies in patients with obesity treated with GLP1RA [4–6]. The authors could not explain the unexpected reduction in total energy expenditure without weight loss in patients with HO, despite they controlled for physical activity, which remained unchanged during treatment.

This is a well-designed study that investigated the effects of GLP1RA in patients with HO on energy balance using the entire spectrum of currently available methods to assess energy intake, free-living energy expenditure, hyperphagia, and hunger and satiety sensations. Future studies are needed to explain the observed reduction in total energy expenditure without observing weight loss during GLP1RA treatment in patients with HO. This research will help to understand the underlying biological mechanisms of energy balance and hunger and satiety regulation in patients with HO.

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11.15. Impaired brain satiety responses after weight loss in children with obesity

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Doi: 10.1210/clinem/dgac299.
https://pubmed.ncbi.nlm.nih.gov/35544121/

Brief Summary: In this intervention study, the effect of a 24-week family-based behavioral obesity intervention on brain activation, measured by visual food cues via functional magnetic resonance imaging (fMRI), and on gut hormone levels was investigated in 9 to 11 year old children with obesity ($n = 28$) and children with normal weight without intervention ($n = 17$). Greater reductions in BMI z-score were associated with a weaker central satiety response, as demonstrated by lesser reductions in meal-induced brain activation to high- vs low-calorie food cues across appetite-processing brain regions from before to after obesity intervention. In contrast, peripheral gut hormones (leptin, ghrelin, peptide YY and glucagon-like peptide 1) improved in individuals with greater reductions in BMI z-score, suggesting an intact peripheral satiety response.

fMRI studies in adults have shown that an increased brain activation to food cues after significant weight loss is associated with weight regain [1, 2]. This study provides important information to understand the underlying mechanisms of dysregulated central and peripheral energy homeostasis. Knowledge about these mechanisms will allow us to develop specific drugs to improve weight loss and prevent future weight regain in children with obesity. However, the results should be interpreted with caution due to the small sample size and the clinically small change in BMI SDS (-0.20 ± 0.22), raising the question if this change is sufficient to cause definite changes in the central satiety response. In addition, the comparison with normal-weight children is difficult to interpret because it is currently unknown whether the different brain responses in children with normal weight and obesity are acquired or inherited, and whether the disturbed brain responses in children with obesity are reversible. Future studies with larger cohorts are therefore needed to investigate the changes in brain activity in children with obesity and find out who responds and who does not respond to obesity interventions.

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11.16. Exenatide for weight-loss maintenance in adolescents with severe obesity: A randomized, placebo-controlled trial

Fox CK, Clark JM, Rudser KD, Ryder JR, Gross AC, Nathan BM, Sunni M, Dengel DR, Billington CJ, Bensignor MO, Kelly AS
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Obesity (Silver Spring) 2022, **30**(5):1105–1115.
https://doi.org/10.1002/oby.23395

Brief Summary: This 52-week randomised placebo-controlled trial showed that exenatide XR improves stabilisation of BMI reduction after lifestyle intervention in adolescents with obesity.

Maintaining weight loss after an intensive lifestyle intervention is usually unsuccessful, especially in adolescents with obesity. In addition to genetic factors with variable effects, factors of living conditions and

individual self-control, there is also a physiological increase in the natural course of BMI values in adolescence. Studies investigating the maintenance of BMI reduction after intervention are of particular importance.

The use of incretin mimetica, in this case the long-lasting GLP-1 receptor agonist exenatide XR, is currently an innovative and effective approach in the pharmacological therapy of obesity. Although there was a clear effect of pharmacological treatment, it did not reach statistical significance. In future, larger numbers of cases should be included in studies. It is possible that more potent incretin mimetica will deliver better results.

The variability of the BMI trajectories after initial weight loss was very high. We do not yet understand the reason for this. The high heterogeneity in the course of BMI shows that it is useful to identify prognostic factors for the therapeutic response in future studies. These might primarily be genetic factors.

To our knowledge, this is the first RCT to specifically target pharmacological interventions for weight maintenance after weight loss in obese adolescents. Special mention should also be made of the fact that there were only mild side effects which were comparable in the intervention and control groups.

11.17. Critical review of bariatric surgical outcomes in patients with Prader-Willi syndrome and other hyperphagic disorders

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Obesity (Silver Spring) 2022, **30**(5):973–981
https://doi.org/10.1002/oby.23385

Brief Summary: This review analyzes the outcomes after bariatric procedures among patients with Prader-Willi syndrome (PWS), melanocortin 4 receptor (*MC4R*) mutations, Bardet-Biedl syndrome (BBS), and hypothalamic obesity (HO). The benefit of bariatric surgery in patients with hyperphagic disorders is only temporary or even absent, indicating the strength of perturbation in the regulation of energy homoeostasis in these patients leading to an elevated body weight set-point [9].

This study is of high interest, since patients with genetic or acquired hyperphagic disorders show an early and rapid increase in BMI accompanied by a high risk of weight-related comorbidities [1,2] but classical weight-management strategies as well as pharmaceutical interventions often fail [2,3].

By reviewing 54 publications from 1974 to 2020 with a total of 202 patients ($n = 114$ with PWS, $n = 43$ with MC4R mutations, $n = 7$ with BBS and $n = 38$ with HO), they identified a notable weight loss in PWS patients over all types of bariatric surgery of -24% within 2 years after surgery. However, this was followed by weight regain beginning 3 years after surgery leading to a non-significant weight-change percentage between 0 and 5 years along with a reoperation rate of 12.5% and a death rate of 9.6%.

Patients with *MC4R* mutations had suboptimal weight loss after gastric bypass [4], weight regain [5] and an increased risk of reoperation [6], whereas patients with HO showed no weight loss after sleeve gastrectomy ($n = 11$), or BMI-decline ($n = 1$), but also band slippage/weight gain ($n = 8$) after band placement [7,8].

This important review shows that also bariatric surgery is not a promising therapy option for patients with hyperphagic disorders, because of high complication rates and suboptimal effectiveness. The satiety impairment due to alterations in hypothalamic signaling pathways, and also modified ghrelin levels in PWS patients is very robust, therefore also behavioral therapy often fails [10].

At the end, obesity in patients with hyperphagic disorders must be accepted as a chronic disease, comorbidities have to be controlled and treated, and aim of the treatment should be a good quality of life. In addition, potent pharmaceutical interventions are needed to overcome these robust mechanisms.

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12. Type 2 Diabetes, Metabolic Syndrome and Lipid Metabolism

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Preface

Globally, new incidences of type 2 diabetes (T2DM) in children and adolescents were estimated as 41 600 in 2021. The first report of a cohort of prepubertal children with T2DM confirms increased prevalence of severe obesity and insulin resistance. The spreading of T2DM worldwide and in young children is seriously alarming. Thirteen years, on average, after initial diagnoses of T2DM, incidence rates for hypertension, dyslipidemia, diabetic kidney disease and retinopathy were more than 50%. A call to remove the term *prediabetes* and to include current glycemic thresholds for prediabetes as *diabetes* may promote early lifestyle and medication interventions.

Of utmost importance for clinical care is the evidence that high BMI, hyperinsulinemia and other metabolic syndrome (MetS) markers are associated with childhood sexual, physical and psychological abuse. Serum ferritin level was identified as a laboratory marker associated with the MetS. Contributions of fructose to obesity, the MetS, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease (CVD) and cancer were reviewed. A number of new drugs, such as glucagon-like peptide 1 receptor (GLP1R) agonist and SGLT2 inhibitors, were shown to improve liver function, and other new drugs showed promising results. New drugs were also developed to treat hypertriglyceridemia, A potent small molecule targeting HNF-1α may be a new therapeutic strategy, and a novel vaccine given every six months proved efficient in mice.

New Data on the Epidemiology of T2DM in Children

12.1. Worldwide estimates of incidence of type 2 diabetes in children and adolescents in 2021

Wu H, Patterson CC, Zhang X, Ghani RBA, Magliano DJ, Boyko EJ, Ogle GD, Luk AOY

Diabetes research and clinical practice 2022;185:109785.

DOI: 10.1016/j.diabres.2022.109785

Brief Summary: This systematic review estimated 41 600 new incident cases of T2DM in children under age 20 years globally in 2021.

Comment: Worldwide estimates of T2DM incidence in children and adolescents under 20 years, both at regional and national levels, were reached by a systematic review and ecological modeling analysis. The latter entails the construction and analysis of mathematical models of ecological processes, either analytic or simulation-based, that predict possible changes in real ecosystems. The association between the risk of T2DM and obesity was confirmed. The highest T2DM incidence was observed in Iran and the lowest in Germany. Considering population sizes, the top three countries with the highest estimated incidences were China, India and the United States of America. The Western Pacific was the region with the highest estimated new incidences, accounting for one-third of the global incidence of T2DM, followed by Africa.

The 41 600 new incidences of diagnosed T2DM in children and adolescents are equivalent to 32% of the estimated incidence of type 1 diabetes (T1DM) in the same age group according to the International Diabetes

Federation Atlas. If undiagnosed T2DM cases were also considered, the number of T2DM in children and adolescents would be substantially greater.

Although this epidemiological study has some limitations due to the paucity of studies and their heterogeneity, the numbers are frightening, especially considering the evidence of early onset of complications in young people, see paper 12.3 (DOI: 10.1056/NEJMoa2100165) in this chapter.

12.2. Type 2 diabetes in prepubertal children

Astudillo M, Tosur M, Castillo B, Rafaey A, Siller AF, Nieto J, Sisley S, McKay S, Nella AA, Balasubramanyam A, Bacha F, Redondo MJ

Pediatric diabetes 2021;22(7):946-50.

DOI: 10.1111/pedi.13254

Brief Summary: This retrospective study describes the characteristics of 35 prepubertal children diagnosed with T2DM during 2016–2019 in Houston, Texas. These children had severe obesity, insulin resistance and dyslipidemia.

Comment: We were all appalled when the first incidence of T2DM in a 5-year-old girl was reported in 2014.[1] However, the situation is even more serious than it appeared then. This is the first report to focus on prepubertal children with T2DM; the mean age was 10.6 ± 2.5 years. These children had severe obesity, acanthosis nigricans and hyperinsulinemia. At presentation, 20% had NAFLD, 14% had hypertension and 5% microalbuminuria. They accounted for 10% of pediatric patients diagnosed with T2DM in a diabetes clinic in Texas. Such early-onset of T2DM is a huge concern, in view of the accelerated development of microvascular morbidity.

One-third of the patients were younger than ten years old! The authors point out that the findings may change the recommendation of the American Diabetes Association (ADA) to screen for T2DM only in children with obesity and other high-risk factors after the onset of puberty or age >10 years, whichever occurs earlier. Furthermore, as treatment with liraglutide, dulaglutide and exenatide have been approved for ages 10 years and older, drug companies should examine the possibility of extending the indications to younger ages.

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New Data on Complications of Children with T2DM

12.3. Long-term complications in youth-onset type 2 diabetes

TODAY Study Group, Bjornstad P, Drews KL, Caprio S, Gubitosi-Klug R, Nathan DM, Tesfaldet B, Tryggestad J, White NH, Zeitler P.

The New England Journal of Medicine 2021;385(5):416-26.

DOI: 10.1056/NEJMoa2100165

Brief Summary: This multicenter observational study assessed the cumulative incidence of diabetic complications in 500 adolescents with Type 2 diabetes (T2DM), followed from 2011 to 2020. After only 13 years from T2DM diagnosis, higher complication rates were observed than those reported for pediatric patients with T1DM or for adults with T2DM.

Comment: This is the second phase of the TODAY study. After completing the first phase, 500 adolescents were transitioned to metformin with or without insulin and were enrolled in an observational follow-up study for over one decade. The first striking finding is the poor glycemic control of the participants. Whereas glycemic control in adolescents with T1DM tends to improve with age, a gradual deterioration occurred in adolescents with T2DM throughout the follow-up; about 45.0% had HbA1c of at least 10% and about 20% had HbA1c of 8–10%. Their median BMI remained 35.0 to 37.5 kg/m$^{2.}$

Poor glycemic control and obesity were associated with early complications. Thirteen years (on average) after initial diagnoses of T2DM, incidence rates for hypertension, dyslipidemia, diabetic kidney disease and neuropathy were 67.5%, 51.6%, 54.8% and 32.4%, respectively, and retinopathy was present in 51%. One-fifth (21.3%) had two complications, and 7.1% had three. Seventeen serious cardiovascular events occurred (four myocardial infarctions, six congestive heart failures, three cases of coronary artery disease and four stroke events). Six deaths were reported. This rapid development of complications was associated with severe insulin resistance and poor socioeconomic circumstances. Hopefully, with the arsenal of new medications for adolescents with T2DM,[1,2.3] we will see a different picture over the next decade.

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12.4. Insulinopathies of the brain? Genetic overlap between somatic insulin-related and neuropsychiatric disorders

Fanelli G, Franke B, De Witte W, Ruisch IH, Haavik J, van Gils V, Jansen WJ, Vos SJB, Lind L, Buitelaar JK, Banaschewski T, Dalsgaard S, Serretti A, Mota NR, Poelmans G, Bralten J.

Translational psychiatry 2022;12(1):59.

DOI: 10.1038/s41398-022-01817-0

Brief Summary: This population genetics study explored pairwise genome-wide genetic correlations between neuropsychiatric disorders with insulin-related somatic diseases and traits. There were likely protective effects of obsessive-compulsive disorder and anorexia nervosa on the risks of having MetS, obesity and T2DM; in contrast there were positive effects of attention-deficit/hyperactivity disorder (ADHD) and major depressive disorders on these metabolic conditions.

Comment: Increased prevalences of insulin-related diseases, namely obesity, the MetS and T2DM, are observed among individuals with neuropsychiatric disorders. Epidemiological data are strongest for Alzheimer's disease, autism spectrum disorder and obsessive-compulsive disorder (OCD). Insulin resistance, a core feature of the MetS and T2DM, is emerging as a potentially important feature in neuropsychiatric disorders. Indeed, insulin receptors are expressed in all cell types in the brain, with widespread distribution of these receptors. This suggests that insulin signaling has essential and diverse roles in the brain.

This genetic study investigated the contributions of insulin resistance-related traits and diseases to neuropsychiatric diseases such as Alzheimer's disease, autism spectrum disorder, obsessive-compulsive disorder, and ADHD, anorexia nervosa, bipolar disorder, major depressive disorder and schizophrenia. Two clusters of neuropsychiatric disorders and insulin-related traits were detected. One was a likely protective effect of the underlying genetics in OCD and anorexia nervosa on the chances of having the MetS, obesity and T2DM. On the other hand, a positive genetic overlap was observed of insulin-related diseases and traits with ADHD.

Interestingly, no correlations were observed of Alzheimer's disease with somatic insulin-related diseases. For Alzheimer's disease, this may suggest a predominant influence of environmental and epigenetic factors in the comorbidity observed in epidemiological studies rather than genetic factors.

12.5. The time is now for new, lower diabetes diagnostic thresholds

Schwartz SS, Rachfal AW, Corkey BE

Trends in endocrinology and metabolism. 2022;33(1):4-7.

DOI: 10.1016/j.tem.2021.10.007

Brief Summary: In this perspective, the authors propose to remove the term *prediabetes* and shift the paradigm to include current glycemic thresholds for prediabetes as *diabetes*.

Comment: In 2001, a group of experts defined the term *prediabetes* to raise awareness of the increasing epidemic of individuals with obesity who had a slight elevation in blood glucose levels. Initially, there was opposition to this term, and it was considered "scaremongering". Although the term was eventually accepted, its definition has changed over time, and there is no consensus regarding the criteria for identifying individuals at the highest risk of progressing to overt diabetes.

Currently, the definition of prediabetes comprises heterogeneous tests of impaired fasting glucose, impaired tolerance to oral glucose, and elevated HbA1c, for identifying individuals at high risk of developing diabetes within five years. Furthermore, the cutoffs of abnormal fasting glucose differ; for example, the World Health Organization (WHO) criterion is 110 to 125 mg/dl and the ADA is 100 to 125 mg/dl.

In this paper, the authors state that current thresholds for diagnosing diabetes are outdated and do not represent advances in disease understanding or the ability to impact its course. They argue that prediabetes is associated with micro- and macrovascular complications, and that lower diabetes diagnostic criteria should be established. This will enable clinicians to prescribe approved antidiabetic agents for more patients. Importantly, early treatment could improve health by mitigating serious complications.

Of note, the definition of T2DM has also changed over the years. In 1985, WHO defined diabetes by either a fasting plasma glucose concentration of ∼140 mg/dl or a glucose concentration of ∼200 mg/dl at 2 h after an oral glucose challenge. In 1997, new recommendations classified diabetes mellitus by a lower cutoff for fasting plasma glucose, of 126 mg/dl. According to the current paper, diabetes should be defined by a fasting glucose concentration above 100 mg/dl.

Expanding the definition of diabetes would have major financial consequences for companies that conduct lab tests, drug companies, device and app developers, clinics, physicians, hospitals, insurance companies and state-funded health provision. Currently, no drugs have been explicitly approved for prediabetes, meaning that we are limited to prescribing diabetes drugs or other medications 'off-label' to treat it. Nevertheless, drug companies are testing dozens of drugs aimed at prediabetes in the hope of tapping a potential worldwide market of hundreds of millions of people. On the other hand, others view that many individuals with prediabetes would be better off untreated (See 10.1126/science.aax2208).

Metabolic Syndrome

12.6. Abuse in childhood and cardiometabolic health in early adulthood: evidence from the Avon longitudinal study of parents and children

Goncalves Soares A, Zimmerman A, Zammit S, Karl A, Halligan SL, Fraser A
Journal of the American Heart Association 2021;10(24):e021701.
DOI: 10.1161/JAHA.121.021701

Brief Summary: This population-based birth cohort study identified an association between childhood abuse and higher BMI at age 18 and 25 years, and also hyperinsulinemia and other markers of the MetS at age 25.

Comment: Previous studies reported that about one-third of adult candidates for bariatric surgery with extreme obesity had undergone sexual abuse. A relation between early life abuse and gestational diabetes has also been reported. Further, an increased risk of T2DM was described among middle-aged women according to the degree of physical and sexual abuse in childhood and adolescence. Additionally, several studies have reported associations of childhood abuse with increased risks of parameters of the MetS in adulthood. However, this subject is so important that it deserves repeated discussion and increased awareness.

This study is part of a population-based birth cohort. A questionnaire that included the three types of abuse (i.e. physical, sexual and psychological) was completed by 3921 individuals. Physical abuse was reported by 5.0%,

mostly before age 11 years, sexual abuse by 12.0% of females and 3.4% of males, and psychological abuse by 13.7% of females and 10.2% of males. Different types of abuse commonly co-occurred. All three types of abuse were associated with higher BMI at age 18 years, and increased frequency of abuse occurrence was associated with higher BMI. At age 25 years, all types of abuse were also associated with higher insulin levels. The age when the abuse occurred was not associated with cardiometabolic outcomes.

Possible explanations for the association between childhood abuse and cardiometabolic health include emotional eating, obesity and direct physiological changes resulting from the stress response system. The key message from this article to pediatricians is the importance of awareness of the possibility of any type of abuse among children with obesity.

12.7. Serum ferritin and the risk of metabolic syndrome: a systematic review and dose-response meta-analysis of cross-sectional studies

Zhang WCB, Xing Y, Shao B.

Biomedical and Environmental Sciences, 2021, 34(8): 623–631

DOI: 10.3967/bes2021.086

Brief Summary: This cross-sectional study identified associations between serum ferritin levels with dyslipidemia and the MetS in both adult men and women. In addition, significant associations were found for ferritin levels with the risks of insulin resistance, prediabetes and T2DM in women.

Comment: Ferritin is a protein that is essential for various vital body organs, processes, functions and diseases. It is particularly needed for iron storage and supply. Obesity is a risk factor for iron deficiency anemia. However, BMI has been shown to be negatively associated with serum iron in adolescents with overweight/obesity but positively associated with plasma ferritin. Thus, circulatory ferritin does not reflect iron deficiency in obesity. Serum ferritin is an acute-phase reactant protein, similar to C-reactive protein, which increases in response to inflammatory processes in people with obesity, including the release of adipokines by adipocytes. Therefore, it is possible that inflammation-induced obesity results in iron deficiency, with elevated iron levels. Elevated serum ferritin levels have been associated with an increased risk of myocardial infarction malignancy and are directly involved in sideroblastic anemias, neurodegenerative disorders, inflammation and hemophagocytic syndrome.

For both genders in this study, the probability of having dyslipidemia increased with serum ferritin levels, suggesting that ferritin affects pathways involved in lipid metabolism. Ferritin may block apolipoprotein B secretion, leading to the accumulation of cellular triglyceride (TG). Ferritin functions as a direct mediator of the immune system, increasing the production of some proinflammatory mediators; chronic inflammation is considered one of the primary mechanisms underlying the MetS. Indeed, in both genders, the probability of having the MetS was also shown to increase as serum ferritin increased. In addition, ferritin can actively participate in the generation of reactive oxygen species. Excessive reactive oxygen species can reduce insulin receptors' affinity, which affects the level of insulin delivery in the muscle and liver. Ferritin was associated with hyperglycemia in women only, independently of age, BMI, and liver and renal factors.

This study adds to previous findings on serum ferritin and the prevalence of the MetS and its components, showing a linear dose–response relationship and different risks between the sexes.

12.8. "Sweet death": fructose as a metabolic toxin that targets the gut-liver axis

Febbraio MA, Karin M.

Cell metabolism 2021;33(12):2316-28.

DOI: 10.1016/j.cmet.2021.09.004

Brief Summary: This paper reviews the links between fructose consumption and health, and the mechanisms by which fructose may damage health.

Comment: Fructose is a plant-derived monosaccharide. Its natural form is found in certain fruits and vegetables. High-fructose corn syrup (HFCS) is a liquid fructose–glucose sweetener produced from corn that has been milled into corn starch, then turned into corn syrup. Then glucose isomerase enzymes are added to convert some of the glucose in the corn syrup into fructose. "HFCS 42" and "HFCS 55" refer to dry weight fructose compositions of 42% and 55%, respectively, the rest being glucose. HFCS commonly substitutes for sugar in processed foods (HFCS 42) and soft drinks (HFCS 55).

Whereas most postprandial glucose from dietary intake is metabolized in peripheral tissues, leaving little for storage as fat in the liver, fructose metabolism is not regulated by insulin or hepatic energy needs. Unlike glucose, most fructose is metabolized and stored by the liver, with little metabolism in peripheral tissues. Fructose also induces de novo lipogenesis, resulting in hepatic fat accumulation and subsequently elevated plasma triglyceride (TG) concentrations. Further, excess fructose consumption disrupts gut barrier integrity, resulting in systemic endotoxemia and leading to the inflammatory cascade and hepatosteatosis.

Evidence is presented that high fructose intake can cause hypertension and hyperuricemia, as well as increased CVD. Data are reviewed on a possible association between fructose consumption and cancer, mainly pancreatic cancer and hepatocellular carcinoma. A critique of this evidence is that results cannot be generalized to the effects of HFCS because pure fructose was investigated at very high concentrations.[1]

Reference

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12.9. Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus

Ferguson D, Finck BN

Nat Rev Endocrinol 2021;17(8):484-95.
DOI: 10.1038/s41574-021-00507-z

Brief Summary: This article reviews the results of clinical trials for modulators of glucagon-like peptide-1 (GLP1) activity, insulin-sensitizing thiazolidinediones, and inhibitors of the sodium-glucose cotransporter 2 (SGLT2) for the treatment of NAFLD and type 2 diabetes mellitus.

Comment: NAFLD has become the most common chronic liver disease in childhood, due to the growing pandemic of pediatric obesity. The current prevalence of NAFLD in the general pediatric population ranges between 3 and 10%, and is up to 50% of children and adolescents with obesity. NAFLD is closely linked to obesity and tightly interrelated with insulin resistance, the MetS and T2DM. Hepatic fat accumulation represents the hallmark of NAFLD, which may progress from simple steatosis through non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis. The first part of this article reviews the results of anti-diabetic agents in NAFLD, and the second part describes experimental agents that have progressed to clinical trials.

There is currently no licensed drug therapy for NASH or NAFLD. Given the tight link between T2DM and NAFLD, numerous anti-diabetic drugs have been tested as treatments:

- Metformin, the first-line T2DM therapy, has not been shown to have beneficial effects on NASH.
- GLP-1 agonists reduce hepatic steatosis and markers of liver damage in genetically induced and diet-induced animal models. Treatment with GLP-1 agonists in individuals with T2DM is effective to reduce hepatic lipid content, liver enzymes and inflammatory markers; the effect is associated with improved HbA1c levels and weight loss. Further, GLP-1 agonists resulted in resolution of NASH with no worsening of fibrosis.
- Sitagliptin, a DPP4 inhibitor, failed to improve hepatic lipid content, despite improvement in HbA1c. Therefore, DPP4 inhibitors might not be an effective strategy for treating patients with NAFLD.
- SGLT2, a sodium-dependent glucose transporter primarily expressed in the proximal tubule epithelium of the kidney, is responsible for most filtered glucose reabsorption. SGLT2 inhibitors increase urinary excretion of glucose and, in individuals with NAFLD, improve hepatic lipid content, liver enzymes and liver stiffness.

The review also discussed the following experimental treatment agents: PPAR agonists, the farnesoid X receptor (FXR) agonist, thyroid hormone receptor beta agonist, fatty acid synthase (FAS) inhibition, stearoyl-CoA desaturase 1 (SCD1) inhibition, diacylglycerol acyltransferase (DGAT) inhibition, and also *FGF19* and *FGF21*.

12.10. Atlas of exercise metabolism reveals time-dependent signatures of metabolic homeostasis

Sato S, Dyar KA, Treebak JT, Jepsen SL, Ehrlich AM, Ashcroft SP, Trost K, Kunzke T, Prade VM, Small L, Basse AL, Schönke M, Chen S, Samad M, Baldi P, Barrès R, Walch A, Moritz T, Holst JJ, Lutter D, Zierath JR, Sassone-Corsi P.
Cell metabolism 2022;34(2):329-45.e8.
DOI: 10.1016/j.cmet.2021.12.016

Brief Summary: In a mouse model, time of exercise during the day was found to determine the magnitude and type of metabolic response. Different tissues responded to exercise at different times of the day.

Comment: Exercise is considered a vital intervention in the prevention and treatment of individuals with chronic and metabolic diseases. However, a growing body of evidence shows that exercise has different results at different times of the day, among different populations. Furthermore, the optimal timing of exercise depends on whether the aim is to improve muscle strength, improve glycemic control, reduce weight loss or reduce depression.

Circadian clocks orchestrate biological processes, including hormone production, behavior and metabolism. Disruption of the circadian clock causes metabolic diseases. While the light/dark cycle has a powerful effect on the circadian clock, feeding and exercise have been shown to reset the circadian clock.

To better understand outcomes of exercise at different times of the day, healthy male mice performed 1-h treadmill exercise either in the early light/rest phase (0900 h) or in the early dark/active phase (2100 h), and were compared to mice who were resting during these times. To understand the exercise-dependent signaling, biochemicals, serum, skeletal muscle, liver, heart, hypothalamus and adipose tissue were collected, and metabolites assessed. Of note, mice are nocturnal. A number of important insights were found:

- Different tissues respond to exercise at different times of the day. For example, exercise at night altered 197 muscle metabolites, whereas daytime exercise impacted only 52 metabolites.
- Responses of different tissues connect and induce an orchestrated adaptation to control systemic energy homeostasis.
- Morning workouts tended to signal greater reliance on fat than sugars to fuel early exercise.
- Late-day workouts might be better for blood-sugar control.

A limitation of the study is it mainly examined a single session of moderate aerobic exercise in male mice. Nonetheless, the notion that exercise has different impact at different times of day and that we can time workouts to achieve specific health goals is intriguing.

Hyperlipidemia

12.11. Identification and evaluation of a lipid-lowering small compound in preclinical models and in a Phase I trial

Wang J, Zhao J, Yan C, Xi C, Wu C, Zhao J, Li F, Ding Y, Zhang R, Qi S, Li X, Liu C, Hou W, Chen H, Wang Y, Wu D, Chen K, Jiang H, Huang H, Liu H.
Cell Metabolism 2022;34(5):667-80.e6.
DOI: 10.1016/j.cmet.2022.03.006

Brief Summary: In preclinical models and a phase 1 trial, a powerful new lipid small molecule was shown to act through a mechanism distinct from those of known hypolipidemic agents. Targeting HNF-1α may be a new therapeutic strategy.

Comment: Familial hypercholesterolemia (FH) is the most common autosomal-dominant genetic disorder. Loss-of-function mutations in the low-density lipoprotein receptor (LDLR) and apolipoprotein B, as well as gain-of-function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9), account for the vast majority of FH cases.

HMG-CoA reductase inhibitors, or statins, are the recommended first-line therapy for most FH patients. However, statin monotherapy is often insufficient to lower LDL cholesterol (LDL-C) to target levels. The addition of a PCSK9 inhibitor (alirocumab and evolocumab) further reduces LDL-C by 43% to 64%. Angiopoietin-like 3 (ANGPTL3) is an endogenous inhibitor of lipoprotein lipase (LPL) and endothelial lipase. A human monoclonal antibody to target and inhibit ANGPTL3 (evinacumab, approved by the FDA in February 2021) reduces LDL-C by up to 23%, and fasting TG levels by up to 76%. This reduction in lipid levels is independent of the LDLR, thus providing hope to individuals with homozygous FH.

This study used an extract from *Corydalis ambigua*, a herb used for centuries in traditional Chinese medicine. They identified a molecule that had a potent lipid-lowering effect both *in vitro* and in animal models. Given its good pharmacological profile and low toxicity, it was selected for a clinical trial. After one month of treatment, serum levels of LDL-C and TG decreased by 19% and 27%, respectively. The molecule downregulated *PCSK9* and *ANGPTL3* transcription by interfering with the binding of HNF-1α to its HNF-1 response element on these two gene promoters. Secondary to *PCSK9* down-regulation, *LDLR* expression was upregulated. Lower *ANGPTL3* expression enhanced lipoprotein lipase activity. Finally, the combination of the new molecule with atorvastatin had an additive effect on lipid clearance, overcoming the elevated PCSK9 levels caused by statin treatment.

Reference

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12.12. Childhood hypertriglyceridemia: is it time for a new approach?

Sunil B, Ashraf AP

Current atherosclerosis reports 2022;24(4):265-75.
DOI: 10.1007/s11883-022-01000-2

Brief Summary: This review article discusses emerging therapies targeted at specific genes, proteins and enzymes to selectively alter triglyceride (TG) metabolism.

Comment: Hypertriglyceridemia is a long-neglected major cardiovascular disease (CVD) risk factor. Recent epidemiological data show that both moderate and severe hypertriglyceridemia are associated with substantially increased cardiovascular disease risk and long-term mortality. This is specifically significant for hypertriglyceridemia beginning in childhood. About 10% of children have circulating TG >150 mg/dl.

Concentrations of fasting TGs are stratified into: normal <130 mg/dl, high ≥ 130–499 mg/dl, very high ≥ 500–999 mg/dl, severe 1000–1999 mg/dl and very severe ≥ 2000 mg/dl. Borderline high TGs are mostly due to acquired factors, such as high carbohydrate intake, obesity, physical inactivity, cigarette smoking, excess alcohol intake and oral hormonal contraception. High values of TGs generally result from a combination of acquired and genetic factors. Very high TGs are typically due to genetic defects. Severe elevation of TG poses a risk of acute pancreatitis, while mild-to-moderate hypertriglyceridemia increases the risk for premature atherosclerotic CVD, and has increasingly been linked with NAFLD.

Indications for genetic testing, dietary recommendations and pharmacotherapy management are detailed in the review. An excellent summary is presented of new drugs that were developed over the last two decades,

including their mechanisms, their effect on lowering TG levels, side effects, ongoing clinical trials in children and FDA/EU approval, as relevant. The new agents include drugs that (1) reduce apolipoprotein C3 [antisense oligonucleotides – volanesorsen and AKCEA-APO-CIII-LRx] and small interfering RNA (siRNA) molecules-AROAPOC3, (2) target ANGPTL3 [ANGPTL3 monoclonal antibody (evinacumab) and GalNac-conjugated antisense oligonucleotide that target ANGPTL3 mRNA in the liver, such as vupanorsen/IONIS-ANGPTL3-LRx] and siRNA AROANG3, or (3) inhibit microsomal TG transfer protein-lomitapide.

In addition to this review, a "Grand Rounds" article in the Journal of Pediatrics[1] describes the clinical presentation, evaluation and management of a 15-year-old male with TG levels >2000 mg/dl.

Reference

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12.13. Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia

Fukami H, Morinaga J, Nakagami H, Hayashi H, Okadome Y, Matsunaga E, Kadomatsu T, Horiguchi H, Sato M, Sugizaki T, Kuwabara T, Miyata K, Mukoyama M, Morishita R, Oike Y

Reports Medicine 2021;2(11):100446.
DOI: 10.1016/j.xcrm.2021.100446

Brief Summary: This mouse study validated a novel, safe treatment with ANGPTL3 peptide vaccine. It was effective to improve obesity-induced dyslipidemia and fatty liver in mice, and also improved dyslipidemia and atherosclerosis in a mouse model of familial hypercholesterolemia, with no reported toxicity.

Comment: Mounting evidence from human genetic studies indicate that elevated plasma TG are a risk factor, in addition to LDL-C, for atherosclerotic CVD.

Angiopoietin-like 3 protein (ANGPTL3) is an inhibitor of both lipoprotein lipase and endothelial lipase in humans. Loss of function mutations in ANGPTL3 are associated with reductions in TG and in non-high-density lipoprotein cholesterol, and reduced risk of coronary artery disease. Therefore, it is logical that ANGPTL3 inhibition could serve as a promising therapeutic strategy to improve lipid levels and lower the risk of CVD. Indeed, anti-ANGPTL3 antibodies, antisense oligonucleotides and siRNAs targeting ANGPTL3 have been developed, and have shown favorable effects on lipid profiles in homozygous familial hyperlipidemia.

These Japanese scientists developed a peptide vaccine that targets ANGPTL3. To this end, they firstly designed a number of ANGPTL3 epitopes as candidate antigens for immunization. They then used these epitope candidates to produce specific antibodies, and selected the one with the best specificity. Next, they explored the effect of ANGPTL3 vaccination on lipid metabolism in ob/ob mice with dyslipidemia. Six weeks after the first immunization, serum TG levels in non-fasting conditions decreased relative to controls. TG accumulation was also lower in the liver of vaccinated ob/ob mice; and steatosis, lobular inflammation and hepatocyte ballooning were alleviated in immunized relative to control mice. ANGPTL3 vaccination showed anti-dyslipidemia effects such as decreasing circulating levels of LDL-C and TG, and anti-atherosclerotic effects, in a severe FH mouse model fed a high-cholesterol diet. The durability of vaccine treatment based on antibody titers against ANGPTL3 persisted until week 30.

As the mouse epitope of the ANGPTL3 sequence is identical to the corresponding human sequence, the vaccine could potentially be investigated in clinical studies in humans. Given that the vaccination is effective for up to 6 months, it may be an outstanding solution.

12.14. Maternal hypercholesterolaemia during pregnancy affects severity of myocardial infarction in young adults

Cacciatore F, Bruzzese G, Abete P, Russo G, Palinski W, Napoli C
Eur J Prev Cardiol 2022;29(5):758-65.
DOI: 10.1093/eurjpc/zwab152

Brief Summary: This retrospective study identified an association between high maternal cholesterol levels during pregnancy with more severe heart attacks in young adult offspring.

Comment: During pregnancy, physiological increases in total cholesterol and triglyceride (TG) levels occur due to increases in insulin resistance, and in levels of estrogens, progesterone and placental lactogen. Maternal hypercholesterolemia, either familial or transient, is associated with early lesions in the fetal aorta. This suggests a role of maternal cholesterol levels during pregnancy on the epigenetic signature in offspring. However, the current treatment of pregnant women with hyperlipidemia consists of diet modification and suspension of lipid-lowering drugs. The long-term effects of maternal hypercholesterolemia on coronary events in offspring are unknown.

This study included 310 hospitalized patients during 1991–2019. Of these, 89 were admitted with a heart attack, at a mean age of 47 years; the 221 hospitalized for other reasons served as controls. Criteria for a severe or non-severe heart attack were the presence of at least one of the following: 1) the number of coronary arteries shows $>75\%$ stenosis; 2) left ventricular ejection fraction $\leq 35\%$; 3) peak levels of creatinine kinase (CK) $>$ 1200 mg/dl and a peak in CK-MB enzymes above 200 mg/dl. Strong correlations were found between each measure of heart attack severity and maternal cholesterol during pregnancy. Further, the maternal cholesterol level was correlated with the number of cardiovascular risk factors, and with the number of cardiovascular risk factors together with clinical manifestations such as heart attack or stroke.

If the findings of this study are confirmed in a larger cohort, measurement of cholesterol levels during pregnancy should be mandatory, and interventions implemented to reduce levels. In addition, affected children should be followed and monitored early.

12.15. Awareness, diagnosis and treatment of heterozygous familial hypercholesterolemia (HeFH) Results of a US national survey

Block RC, Bang M, Peterson A, Wong ND, Karalis DG
J Clin Lipidol. 2021 Sep-Oct;15(5):682-689.
PMID: 34593357
DOI: 10.1016/j.jacl.2021.09.045

Brief summary: This online survey of cardiologists and primary care physicians, conducted by the National Lipid Association, revealed the need to improve awareness, knowledge and treatment of heterozygous familial hypercholesterolemia (HeFH).

Comment: HeFH is an autosomal dominant disorder and the most common inherited cause of premature coronary heart disease. About one in 250 people may have HeFH. In affected persons, atherosclerosis begins at a young age, and untreated children are at a substantially high risk of premature CVD after age 20 years. Even moderate hypercholesterolemia increases the long-term risk of a CVD event. However, huge gaps exist between the progress of science, down to the detail of understanding mechanisms at the cellular and molecular level, the development of advanced drugs to treat hyperlipidemia, and the reality and everyday life.

This online survey of 500 cardiologists and 500 primary care physicians was conducted to assess their recognition of HeFH and its treatment. When asked about the most likely diagnosis of a 30-year-old man with an LDL-C level $>$ 230 mg/dl and a family history of premature CVD, only 57% of cardiologists and 43% of primary care physicians correctly suggested HeFH. Even once recognized, the responses indicated inadequate treatment of HeFH.

Recently, a hybrid model consisting of two existing HeFH criteria coupled with electronic medical record data was suggested to improve HeFH diagnosis and subsequent early access to appropriate treatment[1]. Developing an electronic medical record system with automated laboratory and imaging reports would undoubtedly offer intuitive clinical decision support systems to enable doctors to make better treatment decisions in real-time.

Reference

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12.16. Biased Outcome reporting Guidelines for Underwhelming Studies (BOGUS) statement and checklist

Bauer GR

BMJ 2021; 375:e067350

DOI: 10.1136/bmj-2021-067350

Comment: Do not miss this article from the Christmas edition of *The BMJ* published in 2021.

Short Summary: With some ethanol assistance, the generation of 13 final checklist items and a flow diagram-guided structure of the checklist was produced by a single author who had multiple opinions. These guidelines direct authors to better underemphasize limitations, underestimate bias, and undervalue their readers without underselling their work. The Biased Outcome reporting Guidelines for Underwhelming Studies (BOGUS) initiative fills a gap in the multitude of existing reporting guidelines. While most guidelines (e.g. STROBE, CONSORT, STARD) highlight the strengths of already robust studies, BOGUS highlights the "strengths" of weak studies. Do not miss the visual summary!

13. Global Health for the Paediatric Endocrinologist

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Introduction

For this 7th edition of the global health chapter of the Yearbook for pediatric endocrinology and diabetes, I was happy to see an increasing number of articles discussing direct or indirect access to healthcare. Indeed, in parallel to studies reporting clinical aspects of endocrine conditions and of diabetes that are specific to low- and middle-income countries, there is an abundance of articles that analyze the reasons for poor access to healthcare and propose practical approaches to correct this suboptimal situation. Each of these approaches requires the collaboration of a variety of stakeholders, including pediatric endocrinologists.

Improving Access to Healthcare in Pediatric Endocrinology and Diabetes

13.1. World Health Organization model list of essential medicines for children – 8th list, 2021

World Health Organization, Geneva: 2021
(WHO/MHP/HPS/EML/2021.03)
https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.03

Brief Summary: A major change in the World Health Organization (WHO) 2021 list of essential medicines (EML) is the inclusion, in both the EML for adults and for children, of long-acting analogues of insulin as therapeutic alternatives to human insulin (NPH)

Every 2 years, the WHO publishes a revised List of Essential Medicines for adults (EML, since 1977) and children (EMLc, since 2007). The EMLc applies to children up to 12 years. Human insulin (vials) has been included in the WHO EML since its first edition, in 1977. The most recent EML and EMLc, released in 2021, now include long-acting insulin analogues (insulins detemir, degludec and glargine) for potential inclusion in national EMLs. The addition of long-acting insulin analogues was proposed on several occasions over the last few years, but had always been rejected on the basis of an unfavourable cost benefit ratio. Several reviews have shown modest but definite clinical benefits (i.e. small decrease in HbA1c, decrease in nocturnal hypoglycemia) in patients with Type 1 diabetes, less so in patients with Type 2 diabetes (1, 2). The concern expressed by several experts in the field is that inclusion of long-acting analogues in the WHO EML could make insulin further out of reach for patients in LMICs (3). Indeed, especially in low- and middle-income countries, insulin analogues are much more expensive to the patient than human insulins, despite grossly similar manufacturing costs (4). However, in many high-income countries, where patients can afford insulin analogues, the reality is that pediatric endocrinologists are presently using insulin analogues as first line treatment.

In 2019, the WHO piloted a prequalification program for human insulin, with the hope that it would increase the number of players in the field (beyond the 3 major manufacturers, Sanofi, Novo Nordisk and Eli-Lilly, which together account for $>95\%$ of all insulin prescribed worldwide). Unfortunately, so far, prequalification of human insulin did not attract applications by new manufacturers. In May 2022, the International Consortium for

Pediatric Endocrinology (ICPE, http://intpedendo.org/index.php) and NCD Child (https://www.ncdchild.org/), following a recommendation by Global Pediatric Endocrinology and Diabetes (GPED, *www.globalpedendo,org*), wrote to WHO to request that prequalification of insulin be extended to insulin analogues. This process is now approved!

Finally, it is important to recognize that in parallel to the EML, WHO publishes a List of Essential Diagnostics (EDL). It includes tests that are essential for the management of diabetes and diabetes ketoacidosis, including basic chemistry, HbA1c and glucose monitoring (5).

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2. AA Shafie, CH Ng, YP Tan, N Chaiyakunapruk. Systematic review of the cost effectiveness of insulin analogues in Type 1 and Type 2 Diabetes Mellitus. *Pharmacoeconomics* 2017 Feb;35(2):141–162. DOI: 10.1007/s40273-016-0456-2.
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5. The selection and use of essential *in vitro* diagnostics: report of the third meeting of the WHO Strategic Advisory Group of Experts on In Vitro Diagnostics, 2020 (including the third WHO model list of essential *in vitro* diagnostics). Geneva: World Health Organization; 2021 (WHO Technical Report Series, No. 1031).

13.2. A scoping review of the use of e-learning and e-consultation for healthcare workers in low- and middle-income countries and their potential complementarity

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J Am Medical Informatics Assoc 2022; 29: 713–722.

DOI: 10.1093/jamia/ocab271

Brief Summary: This scoping review found that data remain scarce on the potential synergy between e-learning (EL) training for healthcare workers (HCWs) and provider-to-HCW e-consultation (EC).

Both e-learning (EL) training for healthcare workers (HCWs) and provider-to-HCW e-consultation (EC) have increasing popularity. There are obvious reasons for this, including ease of access (both EL and EC can be enjoyed at any time), virtual interaction at predefined times, lower cost (travel is not needed, decreased cost of conference organisation, no need for printed material), flexibility (can be integrated in a busy schedule) and independence from unpredictable events such as the COVID epidemic, local political situation or worker strikes. However, a key requirements is the use of a digital platform that is suitable for low quality internet and, of course, internet access. These qualities are particularly important for EL and EC in low- and middle-income countries.

Pediatric endocrinologists around the world already have access to EL and EC. Examples of EL include the ESPE e-learning program (https://www.espe-elearning.org/) and the Programme d'Enseignement en Endocrinologie-Diabétologie Pédiatrique pour l'Afrique Francophone (PEDAF, https://e-pedaf.org/site/, supported by the Université Numérique Francophone Mondiale, UNFM, which combines virtual and in-person training and clinical care). Examples of EC include Collegium Telemedicus (https://www.collegiumtelemedicus.org/, supported by Doctors without Borders. Although there is an obvious potential for synergy between EL and EC, Ionescu *et al*. could only identify 3 studies that addressed a variety of aspects of both EC and EL. It is suggested that the combination of EC and EL has the potential to promote collaborations, enhance clinical care and increase the quality of medical training.

13.3. The role of non-governmental organizations in strengthening healthcare systems in low- and middle-income countries: lessons from Santé Diabète in Mali

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Glob. Health Action 2022, VOL. 15, 2061239;
DOI: 10.1080/16549716.2022.2061239

Brief Summary: In this perspective, the authors argue that non-governmental organisations (NGO), such as Santé Diabète, can play a role in the strengthening of health systems and can improve leadership and governance, service delivery and health workforce.

Santé Diabète (SD) is a French NGO dedicated to the improvement of diabetes care in Mali since 2003. It. The authors describe the improvements in diabetes care between 2004 and 2018 and the role played by SD in these successes. They highlight 2 important aspects of their work. First, SD has worked very closely with the Malian health authorities and provided technical expertise for the development of a new strategy on NCDs (national NCD strategic plan). This contributed to the inclusion of many aspects of diabetes care in the plan. Second, SD has taken on many different roles, making their approach to diabetes in Mali very comprehensive. This contrasts with many other NGOs, which mostly focus on advocacy and direct clinical care. Not mentioned in the article but worth mentioning is that the first author (SB) has been present in Mali almost without interruption since the foundation of SD, contributing to the stability of the vision. Interestingly, one of the successes mentioned by the authors is a decrease in the cost of insulin by almost 50% between 2004 and 2018 (expressed in 2018 dollars). While the cost of a vial remains, high, it highlights the importance of a collaboration between SD and the Ministry of Health as well as the complementary experience in getting better contracts with the insulin manufacturers.

13.4. A human rights-based approach to improve access to insulin and other aspects of diabetes care

Brennan F, Williams P, Armstrong K, Klatman E, Donelan N, Ogle GD, Eussen A, Jenkins AJ
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Diabetes Res Clin Pract 2022; 183: 109153.
DOI: 10.1016/j.diabres.2021.109153

Brief Summary: In this perspective, the authors argue that a human rights-based approach to access to insulin empowers patients, families, and communities living with diabetes, and grounds actions by governments, clinicians, and non-government organisations in the principles of dignity, non-discrimination, and equity of access.

Universal health coverage is included under Sustainable Development Goal (SDG) #3 and is one of the overarching targets of the United Nations (UN) 2030 agenda for sustainable development *("Ensure healthy lives and promote well-being for all at all ages")*. However, progress towards this goal, which includes diabetes, remains slow.

Brennan *et al*. take an interesting approach towards access to diabetes management (not only insulin and diabetes supplies but also diabetes care and diabetes information). They explain that access to diabetes care is already codified by international human rights laws (right to health, life, to information and to non-discrimination) and imply that both governments and pharmaceutical companies are expected to respect international laws and obligations. They highlight the guidelines written by the UN Special Rapporteur on the

Right to Health on the expectations from pharmaceutical companies in relation to access to medicines (including insulin). As mentioned in several other articles discussed in this chapter, an important take home message is that progress depends on a joint effort between NGOs, governments and clinicians. Of interest to the reader is also a list of the main international NGOs and other organisations that are contributing to improving access to all aspects of diabetes management, including insulin supply.

13.5. Access to insulin products in Pakistan: a national scale cross-sectional survey on prices, availability, and affordability

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Front Pharmacol 2022 13:820621.
DOI: 10.3389/fphar.2022.820621

Brief Summary: This national cross-sectional survey in Pakistan found poor availability of insulin in the public sector, even for brands manufactured by domestic companies.

Access to insulin remains a major issue worldwide in low- and middle-income countries (LMICs). This survey identifies many of the reasons that prevent access and provides directions for action. First, Pakistan is home to several national biosimilar human insulin manufacturers, including Getz Pharma. The cost of this biosimilar insulin is 20–30% lower than the cost of the originator brand. However, $>$ 90% of the products found in the public sector were manufactured by multinational companies (Eli Lilly, Novo Nordisk and Sanofi), a finding that the authors attribute to marketing and promotional practices. This is an important observation at a time when WHO proposes prequalification of human insulins to facilitate the presence of smaller companies on a larger number of markets. Second, even though the cost of manufacturing human insulin and insulin analogues (recently included in the WHO List of Essential Medicines) is very similar, the retail cost of insulin analogues is 3–4 times higher. One reason may be that Pakistan does not produce biosimilar insulin analogues. Finally, while human insulin is free if obtained in the public sector, it is rarely available, meaning that patients and families have to buy it from the private sector, where it is often unaffordable. Overall, this suggests that policy makers have a major role to play in promoting access to insulin in Pakistan, similar to other LMICs.

13.6. Access to fludrocortisone and to hydrocortisone in children with congenital adrenal hyperplasia in the WHO Eastern Mediterranean region: it takes a village…

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BMJ Global Health 2021;6:e007195.
DOI: 10.1136/bmjgh-2021-007195

Brief Summary: This review found that access to oral fludrocortisone and hydrocortisone remains suboptimal in the WHO Eastern Mediterranean Region. Improvement requires a collaboration between health professionals, families of patients, health authorities, pharmaceutical companies, and the WHO.

This review of access to hydrocortisone (HC) and fludrocortisone (FC), and to newborn screening for congenital adrenal hyperplasia (CAH), in 23 countries of the Eastern Mediterranean Region (EMRO) provides expected but previously undocumented evidence of unacceptable, potentially life-threatening care gaps for children with CAH. Access gaps in the region's 13 low- and lower-middle-income countries (LMIC), such as consistent

unavailability of both FC and oral HC, raise particular concern, including for similar or worse situations in LMICs across all other world regions. The detailed description and analysis of CAH care access gaps discerned by this author group provide first insights and consequently an opportunity to develop first steps towards gap closure. Of particular interest are, first, their documentation that in a region with high rates of consanguinity, and thus about twice higher incidence rates of CAH, universal newborn screening is unavailable except in 2 high-income countries (UAE and Kuwait). Second, availability of oral HC and FC correlates with presence of these medications in National Essential Medicines Lists (NEML; usually templated on the WHO Essential Medicines List that has listed FC and oral and injectable HC since 2009). Third, availability of FC and oral HC correlate with Gross National Income. Fourth, in many countries where access to FC and HC is limited or inconsistent, health care providers and families find creative but temporary and often unsustainable solutions to access medication, such as import by families and friends.

The authors conclude with constructive and realistic potential solutions to the medicine access gap, including the WHO prequalification process, group registration, pooled procurement, working with local pharma companies that will take responsibility for registration and distribution, developing national special access processes, and compounding using active pharmaceutical ingredient – emphasizing that all of the above require collaboration between several stakeholders including patient organizations, pediatric endocrinologists, national health authorities, manufacturers and the WHO.

Footnote: This commentary was kindly prepared by Julia E. von Oettingen, MD, PhD, MMSc, FRCP, Pediatric Endocrinologist, Assistant Professor, Montreal Children's Hospital and McGill University, Montreal, Canada.

Diabetes

13.7. Mortality amongst children and adolescents with type 1 diabetes in sub-Saharan Africa: the case study of the changing diabetes in children program in Cameroon

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Pediatr Diabetes 2022; 23: 33–37.
DOI: 10.1111/pedi.13294

Brief Summary: Despite provision of free insulin by the Changing Diabetes in Children (CDiC) program, mortality remains high in children and adolescents with type 1 diabetes (T1D) in Cameroon, in particular in rural settings and in families with no formal education.

This is the first of two articles on the strengths and weaknesses of the "Changing Diabetes in Children" (CDiC) program, a public-private partnership implemented by Novo Nordisk. It provides free insulin, glucose meters and test strips, as well as basic diabetes education in youth with diabetes and their families. This study describes the characteristics of the 517 patients enrolled in the program in Cameroon between 2010 and 2015. Most patients were enrolled within a few months of their initial diagnosis of T1D. Despite the provision of free insulin and glucose meters, 53 deaths were reported (10% of the patients) over this period. The causes of death remain mostly unknown, but living in a rural setting and lack of formal education were 2 factors significantly associated with mortality.

Overall, this article demonstrates that there is much more to diabetes care than provision of free insulin and blood glucose monitoring. It emphasizes the magnitude of the challenge that families, health professionals and health authorities face in a low-income country to improve diabetes care: understanding the epidemiology and characteristics of T1D in the country, training health professionals, decreasing stigmatization, screening for complications, and advocating for resources are only a few of the areas that come to mind and require urgent attention.

13.8. Improving access to diabetes care for children: an evaluation of the changing diabetes in children project in Kenya and Bangladesh

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Pediatr Diabetes 2022; 23:19–32.
DOI: 10.1111/pedi.13277

Brief Summary: The Changing Diabetes in Children (CDiC) program successfully established a stable supply of free insulin in implementing facilities for children living in Kenya and Bangladesh. However, the program also has limitations, including concerns over its sustainability.

The greatest benefit of the implementation of the "Changing Diabetes in Children" (CDiC) program in Kenya and Bangladesh has been the provision of a stable supply of free insulin for children (although the type of insulin was most commonly human premixed insulin (Mixtard®) which is now rarely used in high-income countries and is not recommended by ISPAD in youth with type 1 diabetes). Other supplies (such as syringes, lancets, glucose strips and glucometers) and education are also offered by CDiC. However, the provision of diabetes care includes many aspects that go well beyond the provision of insulin: dedicated facilities and accessibility to the clinics, staff training and appropriate capacity, education material for patients, availability of supplies and cost of laboratory investigations, to name a few.

The most important message of this article relates to future sustainability of diabetes care in these countries and how the health authorities in Bangladesh and Kenya are planning to progressively decrease their dependence to CDiC. Clearly, the price of insulin, which remains extremely high (see comment on WHO prequalification in paper 13.1 of this chapter) is a major issue. The authors highlight the role that the government is expected to play: care of young adults once they graduate from the program, increased resource allocation to the growing issue of diabetes, better infrastructure, to name a few.

13.9. Global estimates of incidence of type 1 diabetes in children and adolescents: results from the International Diabetes Federation atlas, 10th edition

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Diabetes Res Clin Pract 2022, 183:109083.
DOI: 10.1016/j.diabres.2021.109083

Brief Summary: The incidence of Type 1 diabetes (T1D) in children and adolescents is increasing in many nations. The 10th edition of the International Diabetes Federation Atlas reports that an estimated 108,300 youth aged $<$ 15 years and 149,500 $<$ 20 years were diagnosed with T1D in 2021.

The 10th edition of the International Diabetes Federation Atlas estimates that globally 1,211,900 children and adolescents younger than 20 years have T1D. Comparisons to previous data confirm the steady increase in T1D incidence over the last 20 years. Overall, the numbers show marked differences in the age-standardized incidence between countries. Based on excellent quality data from countries such as Finland (high incidence) and Japan (low incidence), large differences in age-standardized T1D incidence are known to be real. However, in many parts of the world, limitations are present, and many extrapolations were required, all well described in the article.

Looking at the world map that shows the countries with data and how recent the data are, one can easily see that data are unavailable for most countries in sub–Saharan Africa and in Southeast Asia. Data for children aged 0–14 years are based on reports from $<$ 50% of countries and for youth aged 15–19 years from $<$ 15% of countries. Obtaining high quality data from as many countries as possible should be a key priority in order to

understand the epidemiology of T1D diabetes and better advocate for improved management of T1D in youth. There is an opportunity for pediatric endocrinologists working in countries where the characteristics of T1D are poorly documented to advocate for better data.

13.10. Hemoglobin A1c trajectories in the first 18 months after diabetes diagnosis in the SWEET diabetes registry

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Pediatr Diabetes 2022; 23: 228–236.
DOI: 10.1111/pedi.13278

Brief Summary: Analysis of the multi-national SWEET diabetes registry showed that mean HbA1c in youth is highest at diagnosis and lowest at 4 to 5 months post-diagnosis. The HbA1c trajectory was lower in countries with nationalized health insurance. High gross domestic product (GDP) and high use of technology did not seem to protect from a higher trajectory.

To identify characteristics associated with glycemic control in youth with T1D, the authors analyzed data from the SWEET diabetes registry, a multi-national registry of youth with diabetes. 55 centers, divided into 4 regions, participated. Several centers, including India, Mali, Mauritius and Turkey, were located in low- and middle-income countries (LMICs). All centers had one point in common: HbA1c was lowest at 4–5 months after diagnosis. However, several differences between regions are of interest, although the relationship between HbA1c and regional characteristics was not always clear.

First, the region that included countries with the lowest GDP (South America, Asia, Middle East, Africa) also had a lower average HbA1c and a higher insulin total daily dose during the first 18 months compared to Southern Europe, North America, Australia and New Zealand. However, lower GDP was also associated with lower use of insulin pumps and continuous blood glucose monitoring, suggesting that technology per se does not necessarily lead to better metabolic control. In addition, countries with lower GDP were less likely to offer a national health insurance scheme, usually associated with better outcomes. Interpretation was limited by the lack of information on many potential confounding factors, and uncertainty if the participating center is representative of the country? Does the prescribed insulin dose reflect the actual amount used by the patient? Nevertheless, analysis of the SWEET data provides precious information, and it is suggested that more centers in LMICs should join this registry.

13.11. Validation study and outcomes of the diabetes quality of life in youth instrument in Haitian youth with type 1 diabetes residing in Haiti

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Can J Diabetes 2022: 46; 32e39.
DOI: 10.1016/j.jcjd.2021.04.010

Brief Summary: This validation study and survey showed that the Diabetes Quality of Life for Youth (DQOLY) questionnaire and the EuroQol Visual Analogue Scale (EQ-VAS) are valid measures of Health-Related Quality of Life in Haitian youth with type 1 diabetes (T1D).

Tools used for the assessment of the health-related quality of life (HRQOL) in children and adolescents with diabetes are commonly developed in high-income countries. However, it is also important to assess HRQOL in

countries where social characteristics differ markedly from those in high-income countries. This is the case with Haiti, one of the poorest countries in the world. In this study, 20% of patient's parents were not alive, 23% were unable to read or write, and over half did not have a higher than elementary school education. This study validates existing tools, shows that the HRQOL is poor in youth with diabetes in Haiti, and poorer compared to similar studies performed in higher-income countries. Interestingly, metabolic control of diabetes, which is very poor in Haiti, was not a factor in their lower HRQOL. Instead, older age, female sex, and lower socio-economic status were associated with lower HRQOL. One hypothesis might be that the consequences of poor metabolic control are a long-term issue, while QOL reflects immediate concerns, such as poverty and being female. Indeed, other studies have reported a variety of issues perceived by girls with diabetes, such as being a burden on their families, stigma of diabetes care and worries about diabetes affecting their ability to go to school, find a job, get married and bear children. These factors were not studied here but may well exist in Haiti and further emphasize the need for a better, holistic approach to diabetes in Haiti.

13.12. Approach to diagnosing a pediatric patient with severe insulin resistance in low- or middle-income countries

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Clin Endocrinol Metab 2021, 106, 3621–3633.
DOI: 10.1210/clinem/dgab549

Brief Summary: This expert perspective proposes that a thorough review of medical history and physical examination is generally sufficient to diagnose a child with insulin-resistant diabetes mellitus. This simple approach is especially suitable for low- or middle-income countries.

This article emphasizes the role of medical history, careful physical examination, and basic laboratory investigations in making the diagnosis of severe insulin resistance. In low- and middle-income countries (LMICs), patients and families often must pay for investigations (at diagnosis or for follow up) as well as for medicines. This puts them in a tragic situation: no patient should have to choose between getting the right information and getting the right medicine. Unfortunately, most textbooks and review articles are written by pediatric endocrinologists in high-income countries (HICs) and do not take into account the scarcity of resources. The WHO List of Essential Medicines (EML) and List of Essential diagnostics (EDL) (see Paper 13.1 in this chapter) can help identify the medicines and the diagnostic tests that are most cost-effective. In addition, pediatric endocrinologists from HICs and LMICs could jointly write algorithms that highlight the most cost-effective manner to assess and treat a patient. Medical students and fellows in both HICs and LMICs would highly benefit from this approach.

On a different note, the authors found an undetectable insulin concentration in a patient with severe insulin resistance. They hypothesize that "*the low endogenous insulin level can be attributed to the subcutaneous insulin administration 12 hours before the measurement*". This seems unlikely, as the high doses of NPH and of human short-acting insulin administered in this insulin-resistant patient are expected to be detectable in the insulin assay. Another possibility is a technical issue disrupted the assay run. This highlights the fact that, besides their high cost, diagnostic tests need to be reliable.

Endocrinology

13.13. Nutrition in adolescent growth and development

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Lancet 2022; 399: 172–84.
DOI: 10.1016/S0140-6736(21)01590-7

Brief Summary: This review found that the role of nutrition on adolescent growth and development has been poorly studied. Adolescence is a nutrition-sensitive phase for growth, in which the benefits of good nutrition extend to many other physiological systems.

This article is the first of a "Lancet Series" on Adolescent Nutrition. Referrals to pediatric endocrinologists very commonly include short/tall stature, early/delayed pubertal development and under/overweight. While pediatric endocrinologists are trained to identify hormonal issues, they are often not trained on the relationships between nutrition and the epigenetic/hormonal factors underlying these conditions during adolescence. One of the reasons is that the information on this topic is scarce. The authors emphasize that the pubertal transition offers a nutrition sensitive window to promote healthy growth (including body composition, cardiorespiratory fitness, neurodevelopment and immune system development).

They provide several examples of studies performed in low- and middle-income countries on the interaction between nutrition and growth. One interesting example was the negative effect of calcium supplementation on linear growth of boys in Gambia, without benefits on bone mineral content. Overall, they suggest that growth and nutrition should move towards integrated system wide approaches over the life course and that research should lead to a better understanding of the relationships between pubertal development and nutrition, physical activity, and metabolic state. This could lead to novel strategies that optimise growth and prevent non communicable diseases (such as type 2 diabetes, osteoporosis and cardiovascular disease) in later life.

13.14. Invaluable role of consanguinity in providing insight into paediatric endocrine conditions: lessons learnt from congenital hyperinsulinism, monogenic diabetes, and short stature

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Horm Res Paediatr 2022; 95:1–11.
DOI: 10.1159/000521210

Brief Summary: This review highlights the value of consanguinity in the discovery of novel genes for three endocrine conditions with increasing pathophysiological complexity: congenital hyperinsulinism, monogenic diabetes and short stature.

This review nicely highlights that consanguinity has helped pediatric endocrinologists understand the pathophysiology of relatively common conditions. All medical students learn to ask about consanguinity, which is relatively easy to identify through interview of the patient and their family. It is more common in some regions of the world, including Asia, Africa, and the Middle East. Children of parents who are biologically inter-related have areas of genome homozygosity. However, it may be more difficult to identify whether an increased risk of homozygosity is present between parents who are not closely related. Pemberton *et al*. (1) introduced the concept of "runs of homozygosity (ROH)" and developed a population-wide method for identifying genomic segments that are sufficiently long to represent ROH. They devised a model-based clustering scheme that partitions the ROH of a population into three classes: short ROH, resulting from the pairing of ancient haplotypes; intermediate ROH, largely reflecting cryptic relatedness within populations or groups of populations; and long ROH, indicating recent consanguinity. This should be kept in mind when assessing recessive conditions in areas where the gene pool may be limited and when consanguinity between parents does not seem to be present.

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13.15. Comparative effectiveness of East Asian traditional medicine for treatment of idiopathic short stature in children: systematic review and network meta-analysis

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Integr Med Res 11 (2022) 100832.
DOI: 10.1016/j.imr.2022.100832

Brief Summary: This systematic review found that East Asian traditional medicine (EATM) therapies may have beneficial effects in children with idiopathic short stature, however, high-quality studies are necessary.

The authors reviewed 14 studies (1,066 participants) that investigated the effects of East Asian traditional medicines (EATMs) on childhood height. EATMs are widely used to treat various conditions, including idiopathic short stature (ISS). Such treatments include acupuncture, moxibustion (the burning of leaves of mug wort, a small, spongy herb that is believed to enhance acupuncture), herbal medicine, and qigong (a series of exercises that optimize energy within the body, mind, and spirit). Such an analysis is welcome when considering the high cost of growth hormone (GH) therapy, the most commonly used approach for the management of ISS. Unfortunately, most of the studies discussed by the authors were prone to bias in at least some areas, were of short duration (6–12 months) and, with one exception, lacked post-study follow up.

The main conclusion is that EATMS alone may increase height velocity compared to no treatment. The best results were obtained with the combination of GH and herbal remedies (well described in the article). The underlying mechanism of herbal remedies is unclear and might range from a direct effect on growth to indirect improvements in other conditions limiting growth. Despite the many limitations of these studies, it is suggested that the results could serve as a rationale for high-quality studies that formally evaluate the effects of EATMs on growth.

13.16. Gender-role behaviour and gender identity in girls with classical congenital adrenal hyperplasia

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BMC Pediatrics 2021 21:262.
DOI: 10.1186/s12887-021-02742-9

Brief Summary: This questionnaire-based case control study showed that girls with classical congenital adrenal hyperplasia (CAH) had more masculinized gender-role behaviour (GRB) and a tendency for ambiguous gender-identity (GI) compared to healthy children. The most likely contributor was prenatal androgen exposure.

The potential effects of CAH on gender-role behaviour (GRB) and gender identity (GI) have been reported mainly in high-income countries (HIC), with variable findings. Few studies have focused on low- and middle-income countries (LMICs). However, while the prenatal effect of CAH on the brain is expected to be similar in HICs and LMICs, the postnatal characteristics of CAH can be very different between HICs and LMICs: later diagnosis in countries without a neonatal screening for CAH, insufficient availability of hydrocortisone and fludrocortisone (implying suboptimal control of androgen secretion and more frequent central precocious puberty) and more common assignment of male gender in girls with CAH. In addition, cultural and societal norms may also differ markedly between HICs and LMICs.

This study confirms that girls with CAH in Sri Lanka report a more masculinized GRB and a higher tendency for ambiguous GI compared to controls. A limitation of the study may be that the healthy control group comprised 25 girls and 25 boys, and the authors did not mention whether the inclusion of boys as controls influenced the

results. Interestingly, GI and GRB data were unaffected by postnatal factors, such as diagnosis/treatment related factors, suggesting that prenatal androgen exposure was the likely contributor to these outcomes.

The "more mature" reader will remember that, in 1984, Maguelone Forest proposed that prenatal administration of dexamethasone (which crosses the placenta) to the pregnant mother carrying a female fetus at risk of being affected by CAH could decrease virilization of the genitalia and androgenization of the brain. This approach is presently not recommended for various medical and ethical reasons (1), but a reflection on novel, safe approaches aimed at decreasing androgen secretion by the fetal adrenal in female fetuses at risk for CAH could have both physical and behavioural benefits.

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13.17. Health-related quality of life and fatigue perception in children with congenital adrenal hyperplasia: a developing nation perspective

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Pediatr Endocrinol Diabetes Metab 2021; 27: 266–271.

DOI: 10.5114/pedm.2021.109269

Brief Summary: The questionnaire-based case control study found that children with classical congenital adrenal hyperplasia (CAH) in northern India reported lower quality of life (QOL), poorer sleep and increased fatigue compared to healthy peers. Children and parents reported different perceptions of QoL.

We live in a time when major improvements in the diagnosis and management of CAH are taking place: neonatal CAH screening is expanding in many countries, slow-release hydrocortisone is being marketed, CRH receptor inhibitors and gene therapy are being investigated. Sadly, in many low- and middle-income countries, high neonatal mortality, stigmatisation, lack of access to life-saving drugs such as hydrocortisone and fludrocortisone remain highly prevalent, and quality of life (QOL) can be poor.

This study finds a modest decrease in QOL (mainly in the school domain) in patients with CAH compared to age- and sex- matched controls. In contrast, fatigue and sleep were significantly affected. An original aspect of this study is the investigation of fatigue in both patients and parents. The parents of children with CAH reported that the QOL of their affected children was lower and their fatigue greater than the parents of control children. Interestingly, this perception was also worse than the perception of the affected children themselves, emphasizing the negative effect of CAH not only on the children but also on their family. A weakness of the study is the difference in the ratio of boys and girls in the affected group (30/70) and the control group (50/50), contrasting with the mention in the "Methods" section that participants were "Age and sex-matched". Although a greater proportion of girls is expected in the study group (because of the greater mortality of affected baby boys in the absence of a neonatal screening program), this sex difference may confound the overall difference in the perception of QOL and fatigue in the parents of affected and control children.

14. The Year in Science & Medicine

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Preface

A PubMed search for "Medicine and Science" limited to the last 12 months will return 303 153 hits, and if filtered for reviews 38 153. These numbers change to 802 and 127, respectively, when the search is performed with the term "Science and Medicine". This shows that it is exceedingly difficult to search for "the" most important articles to provide in this chapter. Thus we have taken a different approach, by asking members of the team of pediatric endocrinology in Bern to help us in providing their favorite articles in their fields of interest, published in major journals and not already included in other chapters. What resulted is a potpourri of articles covering basic, translation and clinical themes, mostly related to endocrinology. For their efforts in drafting commentaries on their chosen articles, we would like to acknowledge Therina du Toit, Marco Janner, Idoia Martinez de LaPiscina, Emanuele Pignatti, Christoph Saner, and Grit Sommer.

We all hope you enjoy our personal selection of the most important literature published in "Medicine and Science" in the last 12 months.

Basics of Human Biology

14.1. Daily energy expenditure through the human life course

Herman Pontzer *et al.*
Science. 2021 Aug 13;373(6556):808–812
Doi: 10.1126/science.abe5017

Brief summary: This study compiled a large database of doubly labeled water measurements on a large ($N = 6421$) and diverse ($N = 29$ countries) population aged between 8 days and 95 years to provide the first comprehensive study of total energy expenditure (TEE) and basal metabolic rate (BMR) over the human life span.

The results revealed that TEE increased with fat-free mass in a 'power law' manner with four distinct life phases: the first phase is neonates up to 1 year of age. Neonates had similar fat-free-, and fat mass-adjusted TEE and BMR as adults. Until 9–15 months of age, adjusted TEE and BMR both rise to values $\sim$50% above respective adult values. In the second phase, 'juveniles' between 1 and 20 years of age, adjusted TEE and BMR steadily declined to meet adult levels. Interestingly, no puberty-related increase was evident among subjects aged 10–15 years. The third phase is adulthood from 20 years to 60 years with stable adjusted TEE and BMR, even for women during pregnancy. The fourth phase is adults aged >60 years, when adjusted TEE and BMR declined; subjects 90 years and older had levels $\sim$26% below those of middle-aged adults.

The analyses provide empirical measures and predictive equations for TEE and BMR from infancy to senescence. The results shed light on metabolic changes across the life course, they identify critical periods for energy disbalance with respect to growth and development, and they challenge old beliefs: First, adolescents in puberty or women during pregnancy do not show a higher metabolic rate. Rather, it was infants and toddlers who burned the most calories when adjusted for body size, which may reflect elevated energy demands related

to growth and development. Second, neonates revealed the same metabolic rate as their pregnant mothers, which is no different from other women when adjusted for body size. Third, the energy costs of physical activity and of tissue-specific metabolism were not constant throughout life but showed age-dependent differences. The findings highlight the importance of energy requirements during infancy for growth and development. By contrast, your 'middle-age spread' is not due to slowing of metabolism; rather this age-related decline was not apparent until after age 60 years, and so other lifestyle factors are to blame!

14.2. Cholesterol is required for transcriptional repression by BASP1

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Proc Natl Acad Sci U S A. 2021 Jul 20;118(29):e2101671118.
DOI: 10.1073/pnas.2101671118

Brief Summary: This cell model study shows that cholesterol is essential for the transcriptional repressor and cell differentiation activities of brain acid soluble protein 1 (BASP1).

Cholesterol biology covers a spectrum of functions spanning from the biosynthesis of Vitamin D, bile acids and steroids, to the control of plasma membrane fluidity, protein trafficking, and signaling transduction. This study further expands this lengthy list by describing a novel function of cholesterol in the cellular nucleus, where it drives the activation of the transcriptional co-repressor Brain acid soluble protein 1 (BASP1).

BASP1 binds and inhibits the transcriptional activity of Wilms tumor 1 (WT1), a transcriptional factor best known for its association with early onset nephroblastoma. Depletion of BASP1 in adult fibroblasts replaces the requirement of three Yamanaka reprogramming factors for the induction of pluripotency, which underscores BASP1's role in cell differentiation through WT1. The authors use both cell-free and K562 chronic myelogenous leukemia cell line-based assays, combined with immunoprecipitation and immunofluorescence, to demonstrate that BASP1 interacts with cholesterol in cell nuclei through a conserved CRAC motif. Immunodetection in cell nuclei also showed that BASP1 knock-down resulted in almost complete absence of nuclear cholesterol, suggesting nuclear BASP1 is a major reason for cholesterol being in the nucleus. They also found that BASP1 recruits cholesterol to the gene promoter regions of WT1 target genes, and that BASP1-cholesterol interaction is necessary for the control of K562 cell differentiation towards a neuronal-like phenotype. In line with this, the authors show that inhibition of cholesterol synthesis, using atorvastatin, lovastatin, or tripanarol, reduced the phorbol 12-myristate 13-acetate (PMA)-induced differentiation of K562 cells, and disruption of the CRAC domain resulted in complete inhibition of the co-repression function of BASP1.

While clinical attention nowadays focuses on systemic cholesterol metabolism and cytotoxicity of cholesterol excess because of its tight association with atherosclerotic risk, the importance of local cholesterol distribution and cellular functions is mostly overlooked. The work by Loats *et al*. makes a step forward in our understanding of local/cellular functions of cholesterol, by highlighting how cholesterol availability can ultimately impact cellular differentiation.

14.3. Structure and transport mechanism of the human cholesterol transporter ABCG1

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Cell Rep. 2022 Jan 25;38(4):110298.
DOI: 10.1016/j.celrep.2022.110298

Brief Summary: This study describes the cryo-EM structure of human ATP Binding Cassette Subfamily G Member 1 (ABCG1) in complex with cholesterol. The authors propose a structural mechanism whereby cholesterol is recruited from cell membranes by sphingomyelin and loaded on nascent HDL particles.

ABCG1 mediates the addition of cholesterol to nascent HDL particles as part of the reverse cholesterol transport destined to excess cholesterol catabolism and excretion by the liver. This role of ABCG1 is mostly studied in macrophages, where ABCG1 inactivation results in intracellular accumulation of neutral lipids and phospholipids that facilitates the formation of foam cells and atherosclerotic plaques. These authors report the first cryo-EM structure of human ABCG1 at a resolution of 3.26 A ä. They combine *in vitro*and functional assays to propose a model in which ABCG1 recruits 2 cholesterol molecules, each shuttled by a sphingomyelin moiety. Following activation by ATP, cholesterol is then released by the transport protein to acceptor HDL particles through a hydrophobic channel made of 3 pairs of Phenylalanine residues.

This study offers the first structural glance into a key component of the reverse cholesterol pathway. Therefore, it holds strong promise for shedding further light on the mechanisms that regulate physiological extrusion of excess cholesterol from macrophages and prevent their transformation into foam cells and atherosclerotic events.

Steroidogenesis and Beyond

14.4. Quantification of androgens and their precursors in full-term human placenta

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Eur J Endocrinol 2021;185:K7–K11
DOI: 10.1530/EJE-21-0312

Brief Summary: Steroid profiling of 10 placentas of healthy full-term neonates (five male and five female) using liquid chromatography-tandem mass spectrometry revealed the presence of C11-oxy C_{19} steroids (androgens) in the fetal-placental unit at term – pointing to alternative androgen pathways in this unit.

The authors introduced for the first time the relevance of the C11-oxy androgens (also termed 11-oxygenated androgens) in the human placenta, which forms an integral part of the fetal-placental unit. Previously, the adrenal precursor metabolite to C11-oxy androgens, 11β-hydroxyandrostenedione (11OHA4), had been measured only in amniotic fluid between 15–32 weeks post conception [Hampl *et al*., 1990] and biosynthesised by the fetal adrenal by 16 weeks post conception [Villee and Driscoll 1965; Duffer and Villee 1969]. In this study, 11OHA4, together with 11-ketoandrostenedione (11KA4), 11β-hydroxytestosterone (11OHT) and 11-ketotestosterone (11KT) were quantified in term placental tissue taken from the fetal side, in the order of 11KA4 > 11KT > 11OHA4 > 11OHT. The biosynthesis of C11 keto-metabolites in the placenta is expected due to the high expression of placental 11β-hydroxysteroid dehydrogenase type 2 (*HSD11B2*), however unexpectedly, these authors show that the placenta is an androgen producing organ and underscores that the placenta doesn't only shuttle steroids from the maternal unit to the fetal unit and back, but contributes to active steroidogenesis. This study is elegantly complemented by a subsequent study which quantified the C11-oxy androgens in neonatal cord blood, once more reporting high levels of 11KA4 [He *et al*., 2022].

It is worth mentioning that, while 11KT was quantified at lower levels compared to 11KA4, 11KT is a potent androgen and its contribution to human steroid-related diseases and disorders has become increasingly noticeable since 2012. The biosynthesis of this androgen in the fetal-placental unit therefore becomes a focus point for future research endeavours, to determine the role of this androgen in fetal development, with a follow-up research endeavour being unravelling the origin of the C11-oxy androgens in the fetal-placental-maternal unit. Indeed, adrenal 11OHA4 could originate from the fetal adrenal and/or the maternal adrenal (circulation) serving as the precursor to placental 11KA4 and 11KT biosynthesis. As the presence of the C11-oxy androgens has now been established in maternal circulation and in placental and fetal tissues, it becomes clear that the next crucial step will be to determine their impact on human fetal development.

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14.5. Embryonic osteocalcin signalling determines lifelong adrenal steroidogenesis and homeostasis in the mouse

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J Clin Invest 2022;132(4):e153752
DOI: 10.1172/JCI153752

Brief summary: Using rodent and primate models, this study shows a classical endocrine feedback loop, where bone-derived osteocalcin enhances adrenal steroidogenesis, and the inactivation of osteocalcin signalling impairs adrenal growth and steroidogenesis.

This study provides important evidence on the existence of the bone/adrenal axis and its effect on fetal adrenal development and reveals that bone-derived osteocalcin (Ocn) modulates fetal adrenal homeostasis, termed the 'bone-adrenal endocrine circuit' [additional commentary is provided by Dumontet & Hammer 2022]. The authors showed that Ocn injections increased circulatory corticosterone (to a similar extent as observed with adrenocorticotropic hormone) and aldosterone (also observed in rhesus monkeys), together with the upregulation of melanocortin receptor 2 (*Mc2r)* and steroid 11β-monooxygenases, *Cyp11b1* and *Cyp11b2*, expression. Moreover, Ocn-deficient ($Ocn^{-/-}$) mice born from $Ocn^{-/-}$ parents showed lower corticosterone and aldosterone levels and decreased expression of *Cyp11b1* and *Cyp11b2* compared to WT mice and $Ocn^{-/-}$ mice born from a cross between $Ocn^{+/-}$ parents. Ocn signalling during fetal development assisted murine adrenal gland growth, and the authors concluded that embryologic Ocn may regulate adrenal steroidogenesis and adult adrenal function.

The initiation of this study worked in reverse, starting from the question: if glucocorticoids (GC) influence *Ocn* expression, then perhaps Ocn influences GC production. Rewardingly, inferring that Ocn regulates GC biosynthesis established an important link between bone formation and adrenal development, and underscores Ocn signalling as an important factor in the lifelong regulation of adrenal growth and steroidogenesis. As exciting as this research is, further studies now have to prove the relevance of the bone/adrenal axis to human fetal adrenal development and human biology, while the translational potential of targeting Ocn to modulate adrenal function also remains to be elucidated.

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14.6. Altered steroidome in women with gestational diabetes mellitus: Focus on neuroactive and immunomodulatory steroids from the 24th week of pregnancy to labor

Ondéejíková L, Paéízek A, Šimják P, Vejražková D, Velíková M, Anderlová K, Vosátková M, Krejčí H, Koucký M, Kancheva R, Dušková M, Vaňková M, Blunt J, Hill M
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Biomolecules 2021;11:1746
DOI: 10.3390/biom11121746

Brief summary: Comprehensive steroid profiling of pregnant women with and without gestational diabetes mellitus (GDM) during late gestation revealed specific GDM-related steroidomic changes, linking the maternal compartment to GDM pathophysiology and highlighting the interaction between GDM and the stage of gestation.

It is well known that GDM has serious health implications for both the mother and the developing fetus, as it would be expected with induced glucose intolerance leading to persistent hyperglycaemia and insulin resistance that signifies this condition [Plows *et al*., 2018]. This study evaluated the alterations in steroid levels in women with GDM at crucial stages of late gestation and at labor, to better understand the possible hormone role-players involved in neuroactive and immunomodulatory processes. The prelude to this study was the development of an analytical method that quantifies 100 endogenous steroids in human serum [Hill *et al*., 2019] – an impressive task which rewarded the authors with a powerful tool to perform extensive steroid profiling of *in vivo* samples.

The authors found altered levels of 49 steroids between women with and without GDM. These differences show that the pathophysiology of GDM, in terms of steroidomics, is dominant in the maternal compartment compared to the fetal-placental compartment (determined through the analysis of mixed umbilical cord blood). Steroid hormones involved in multiple steroid pathways, together with those reduced by hepatic enzymes were significantly different. In addition, progesterone was confirmed as a pro-diabetogenic steroid, as levels were substantially higher in GDM women, but on the contrary, its levels were lower at labor. These authors complete an in-depth analysis of the biochemistry, entailing the production of the steroid metabolites which were differentially measured and ultimately relate these steroid levels to steroid metabolic enzymes.

Any steroidomic approach to understanding complex steroid metabolic pathways is a large undertaking, and even more so when it is applied to a disease condition. Unfortunately, the lack of sampling before 24 weeks gestation makes it difficult to understand if any changes might contribute to GDM pathogenesis rather than being consequential. However, such investigations are expected to substantially increase in the future as our analytical capabilities improve and will surely provide data from which newer steroid signalling cascades can be modelled and novel steroid biomarkers identified.

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14.7. Placental uptake and metabolism of 25(OH)vitamin D determine its activity within the fetoplacental unit

Ashley B, Simner C, Manousopoulou A, Jenkinson C, Hey F, Frost JM, Rezwan FI, White CH, Lofthouse EM, Hyde E, Cooke LDF, Barton S, Mahon P, Curtis EM, Moon RJ, Crozier SR, Inskip HM, Godfrey KM, Holloway JW, Cooper C, Jones KS, Lewis RM, Hewison M, Garbis SDD, Branco MR, Harvey NC, Cleal JK
The Institute of Developmental Sciences, Human Development and Health, Faculty of Medicine University of Southampton, Southampton, United Kingdom
eLife 2022;11:e71094
doi: 10.7554/eLife.71094

Brief summary: Using placental perfusion, placental fragment culture and primary term human cytotrophoblast culture experiments, the authors signify the relationship between maternal vitamin D, placental vitamin D metabolism and fetal vitamin D exposure.

In this study, the uptake of radio-labelled vitamin D (^{13}C-25(OH)D$_3$) could be elegantly traced from the maternal unit to the placental unit through to the fetal compartment, with the placental unit additionally metabolising and storing maternal vitamin D. Active placental metabolism of maternal vitamin D resulted in the biosynthesis of

downstream C24- and C1-hydroxylated vitamin D metabolites, both of which were released into the maternal and fetal compartments, while these metabolites also remained within the placental pool. Of the two downstream metabolites, C24-hydroxylated vitamin D was predominantly produced in the placenta and two-fold more of this metabolite was released into the maternal unit compared to the fetal compartment, also supported by positive correlations between placental and maternal vitamin D metabolite levels.

Importantly, the authors show that vitamin D uptake is an active uptake mechanism- not by simple diffusion – entailing similar mechanisms of endocytosis as observed in the kidney. This study furthermore shows that exposure of the placenta to vitamin D leads to transcriptomic and proteomic changes in the placenta, which ultimately impact the specificity of the vitamin D response in the fetal-placental unit. Bringing these results into context of the fetus, these findings predict that impaired placental transport and metabolism of maternal vitamin D may hinder fetal development. Indeed, the authors substantiate their conclusions with results from a cohort study, in which placental expression levels of key vitamin D metabolising enzymes and metabolic genes were associated with fetal size measurements.

This is the first quantitative study to demonstrate vitamin D transfer and (most importantly) metabolism by the human placenta. It reveals a complex interplay between vitamin D and the placenta, with ultimate impact on fetal growth and development. These results are important and now the continuing story of the cooperation between vitamin D and the fetal-placental unit needs to be understood within the context of early gestation to fully understand the translational capacity of these results.

Risk and Outcome

14.8. Development over time of the population-attributable risk fraction for cannabis use disorder in schizophrenia in Denmark

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JAMA Psychiatry. 2021 Sep 1;78(9):1013–1019.
PMID: 34287621.
DOI: 10.1001/jamapsychiatry.2021.1471.
https://jamanetwork.com/journals/jamapsychiatry/fullarticle/2782160

Brief Summary: This nationwide prospective registry study from Denmark in >7 million individuals reports that the proportion of schizophrenia diagnoses attributed to cannabis use disorder increased from 2% in 1995 to 8% in 2010.

In the last decades, the use and potency of cannabis products has increased. Daily cannabis use is associated with the development of schizophrenia-related psychoses, but it was under debate whether the association is causal. This nationwide Danish study provides evidence for causality by showing a time trend of increasing schizophrenia attributed to cannabis-use disorder, while considering a wide range of potential confounding factors, including individual and parental alcohol and other substance use disorder, and family history of psychoses. They calculated the 'E-value' to be as high as 5–10, indicating that a potential unmeasured confounder would need to have an unlikely high effect to explain the association between increasing schizophrenia with increasing cannabis use.

Rising incidence of schizophrenia due to cannabis use is likely driven by the increasingly heavy use of high-potency cannabis products, which became available over recent years. Several countries recently legalized cannabis, and many people may misinterpret this decision as meaning that cannabis use is harmless. Educating communities, especially adolescents and young adults, about the dangers of high-potency cannabis is important for preventing psychotic disorders.

14.9. A proteomic surrogate for cardiovascular outcomes that is sensitive to multiple mechanisms of change in risk

Williams SA, Ostroff R, Hinterberg MA, Coresh J, Ballantyne CM, Matsushita K, Mueller CE, Walter J, Jonasson C, Holman RR, Shah SH, Sattar N, Taylor R, Lean ME, Kato S, Shimokawa H, Sakata Y, Nochioka K, Parikh CR, Coca SG, Omland T, Chadwick J, Astling D, Hagar Y, Kureshi N, Loupy K, Paterson C, Primus J, Simpson M, Trujillo NP, Ganz P

Sci Transl Med. 2022 Apr 6;14(639):eabj9625;
PMID 35385337
DOI: 10.1126/scitranslmed.abj9625
https://www.science.org/doi/10.1126/scitranslmed.abj9625

Brief Summary: This international study, led by Dr. Stephens Williams from Boulder, Colorado, USA, developed a surrogate biomarker from 27 circulating proteins to predict the risk of having a cardiovascular disease (CVD) event in the next 4 years.

The study used data from > 30 000 plasma samples of > 20 000 participants from 9 large clinical studies. From a total of 5,000 proteins measured, a machine learning approach selected 27 proteins with high prognostic ability to predict the 4-year likelihood of myocardial infarction, stroke, heart failure, or death. In a validation cohort, the 27-protein model performed twice as well as previous tests, and also performed well in individuals with pre-existing conditions putting them at high risk for CVD events, including individuals with a previous heart attack or stroke, cancer survivors, individuals with diabetes or smokers.

This test can be used as a marker to monitor whether a patient is optimally treated according to their CVD risk profile. It may also help to accelerate the process of drug development, as it would give an immediate measure of health during clinical trials, rather than having to wait in uncertainty for the benefits or risks that may manifest only after long-term treatment.

14.10. Childhood cardiovascular risk factors and adult cardiovascular events

Jacobs DR Jr, Woo JG, Sinaiko AR, Daniels SR, Ikonen J, Juonala M, Kartiosuo N, Lehtimäki T, Magnussen CG, Viikari JSA, Zhang N, Bazzano LA, Burns TL, Prineas RJ, Steinberger J, Urbina EM, Venn AJ, Raitakari OT, Dwyer T
N Engl J Med. 2022 May 19;386(20):1877–1888.
doi: 10.1056/NEJMoa2109191.

Brief summary: This prospective cohort study leveraged data from the International Childhood Cardiovascular Cohorts (i3C) Consortium, including 42 324 participants at baseline and followed-up over a mean of 35 years, in order to investigate associations between cardiovascular risk factors (CVRF, including body-mass index, systolic blood pressure, total cholesterol levels, triglycerides, and smoking) in childhood (mean age 11.8 years) with fatal and non-fatal cardiovascular events in adulthood (mean age 47 years). They showed that increases in each individual CVRF in childhood, and a combined-CVRF risk score, were associated with higher risks of fatal and non-fatal cardiovascular disease events in adults, robust to adjustment for adult CVRF levels.

According to post-mortem pathology studies among youths the process of atherosclerosis starts in very early life and that its severity is associated with both, the presence, and the severity of CVRF. However, direct evidence that CVRF in childhood are linked to hard cardiovascular disease endpoints in adulthood has remained elusive.

The study results showed that hazard ratios for a fatal and non-fatal cardiovascular event in adulthood increased per standard deviation (SD) increase of each individual CVRF, and even more per an increase of a combined-risk z-score, irrespective of race or sex. Adjustment for CVRF measurements in adulthood attenuated the hazard ratio of the childhood combined-CVRF risk score, but the latter remained significant, which highlights the likely direct adverse effects of childhood CVRF on later disease risks. Also, changes in CVRF profiles were associated with fatal and non-fatal cardiovascular disease endpoints.

The results illustrate that both CVRF in childhood and the path to risk in adulthood are important to predict adult cardiovascular disease events. These findings provide robust evidence that reduction in CVRF levels in childhood have the potential to prevent adult incidence of premature cardiovascular events.

14.11. SGA-born adults with postnatal catch-up have a persistently unfavourable metabolic health profile and increased adiposity at age 32 years

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European Journal of Endocrinology (2022)187:15–26
DOI: 10.1530/EJE-21-1130

Brief Summary: This study, PROGRAM32, investigated 287 adults mean age 32 years who had participated 10 years earlier in the PROGRAM study on the metabolic and cardiovascular consequences of low birth weight (LBW). The aim was to investigate the adverse metabolic profile of being small for gestational age with spontaneous postnatal catch-up (SGA-CU) compared to age-matched controls born appropriate for gestational age (AGA), and to characterise cardiovascular health in both groups.

LBW is associated with higher risks for type 2 diabetes and cardiovascular disease in adulthood. In the previous PROGRAM study, SGA-CU individuals at age 21 years showed insulin resistance, higher fat mass and an adverse lipid profile.

The current study shows that, 10 years later at age 32 years, SGA-CU adults had lower insulin secretion, higher fat mass in the trunk and an adverse lipid profile compared to AGA. However, these parameters did not worsen over time. Furthermore, gain in weight-SDS from birth to 32 years was associated with higher total FM and visceral FM as well as a higher risk for developing the metabolic syndrome at 32 years. It should be noted that this study considered 'spontaneous catch-up growth' – by contrast catch-up growth that is induced by growth hormone therapy has been shown to have beneficial metabolic effects (1).

This is the largest follow-up study of long-term metabolic and cardiovascular health consequences of LBW. The authors use high-quality research tools, including frequently sampled i.v. glucose tolerance test, DXA and MRI, to assess the 170 former LBW individuals.

This study adds to previous evidence that the unfavourable body composition and adverse metabolic profiles consequent to LBW persist into adulthood, particularly in LBW individuals who show spontaneous catch-up growth. Further follow-up studies will be necessary to assess cardiovascular endpoints and the impact on incidence of type 2 diabetes.

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14.12. Smoking during pregnancy is associated with child overweight independent of maternal pre-pregnancy BMI and genetic predisposition to adiposity

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Scientific Reports (2022)12:3135

DOI: 10.1038/s41598-022-07122-6

Brief Summary: This case cohort study, based on the Danish National Birth Cohort (DNBC) with ∼100 000 women and their children, investigated whether maternal smoking in pregnancy is associated with child BMI and risk for child overweight, independent of maternal pre-pregnancy BMI, genetic predisposition to adiposity and socio-economic factors. Furthermore, the authors assessed the interaction of maternal predisposition to adiposity on the association between maternal smoking on child BMI.

The study shows that any smoking during pregnancy is associated with higher child BMI at age 7 years. This association was weaker when adjusted for maternal BMI or maternal BMI GRS (genetic risk score) for BMI. Furthermore, smoking during pregnancy was associated with higher odds for childhood overweight in a dose-dependent manner: the odds ratio for ≧ 11 cigarettes/day in the third trimester was 2.42 (1.3–4.5; 95% confidence interval) compared to non-smoking, irrespective of maternal BMI or maternal GRS. In addition, there was no interaction between maternal GRS and smoking. The study is based on the very large Danish National Birth Cohort with more than 90 000 mother-child pairs allowing a unique study design with a follow-up time of 7 years. In addition, maternal genetic predisposition to adiposity was assessed as a GRS, and maternally-transmitted GRS could be analysed separately from non-transmitted (maternally-acting) GRS.

This study adds to previous evidence that the association between smoking during pregnancy and child overweight is not confounded by a genetic predisposition to adiposity of the mother. Furthermore, using a design

with high statistical power, the study confirms that the effects of smoking during pregnancy on childhood adiposity are lasting at least up to 7 years. The mechanisms are unclear, possibly relating to *in utero* exposure on developing adipocytes or appetite-regulatory neurons, or consequent to the early rapid catch-up in growth and weight gain that is typically seen in these infants. However, they add support to public health efforts to eradicate smoking exposure during pregnancy.

New treatments

14.13. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis

Julian D Gillmore, Ed Gane, Jorg Taubel, Justin Kao, Marianna Fontana, Michael L Maitland, Jessica Seitzer, Daniel O'Connell, Kathryn R Walsh, Kristy Wood, Jonathan Phillips, Yuanxin Xu, Adam Amaral, Adam P Boyd, Jeffrey E Cehelsky, Mark D McKee, Andrew Schiermeier, Olivier Harari, Andrew Murphy, Christos A Kyratsous, Brian Zambrowicz, Randy Soltys, David E Gutstein, John Leonard, Laura Sepp-Lorenzino, David Lebwohl

N Engl J Med. 2021 Aug 5;385(6):493–502.
PMID: 34215024
doi: 10.1056/NEJMoa2107454. Epub 2021 Jun 26.

Brief Summary: 6 patients with hereditary, life-threatening transthyretin amyloidosis were treated with a novel *in vivo* gene-editing therapeutic agent (NTLA-2001) based on CRISPR-Cas9 technology. The new agent was given intravenously in a single dose and was able to durably knockout the responsible *TTR* gene and reduce the misfolded protein in the serum in a dose dependent manner over an observation period of 12 months.

Monogenetic transthyretin amyloidosis is caused by more than 100 mutations in the *TTR* gene, which cause the TTR protein to fold in a wrong shape and form clumps/fibers that interfere with organ function, especially in the heart and nerves. The disease is progressive and life limiting. So far, treatment consists of reducing amyloid formation by either stabilizing the tetrameric form of the TTR protein or by inhibiting its synthesis by degradation of the mRNA. However, these treatments are unsatisfactory for several reasons, including efficacy, side effects and need of long-term repeated administration.

So far, clinical trials had shown that the CRISPR-Cas9 technique can edit genes in cells that have been removed from the body and then reinfused back to the patients. This study is the first to show that the technique is safe and effective for treating a monogenetic disorder, when the CRISPR–Cas9 components are infused directly into the patients. Although promising, there are some limitations: Functioning of the technique depends on a DNA-cutting enzyme (Cas9), and a piece of guide RNA targeting the mutated gene. Thus the essential components must be packaged in a specific way that protects them from degradation and delivers them to the correct target body tissue or organ. Techniques for packaging and targeted delivery of CRISPR–Cas9 components to various parts of the body are improving. In the case of transthyretin amyloidosis, where the editing tools are needed in the liver, they were encapsulated into lipid nanoparticles, which are taken up by the liver from the circulation. It will be fascinating to follow how this technology might become applied to the treatment of other genetic conditions, even in later life.

14.14. A novel therapeutic strategy for skeletal disorders: Proof of concept of gene therapy for X-linked hypophosphatemia

Volha V Zhukouskaya, Louisa Jauze, Séverine Charles, Christian Leborgne, Stéphane Hilliquin, Jérémy Sadoine, Lotfi Slimani, Brigitte Baroukh, Laetitia van Wittenberghe, Natalie Danièle, Fabienne Rajas, Agnès Linglart, Federico Mingozzi, Catherine Chaussain, Claire Bardet, Giuseppe Ronzitti

Science Advances, 2021,7:eabj5018
DOI: 10.1126/sciadv.abj5018

Brief Summary: The authors developed a liver-targeting adeno-associated virus (AAV) vector carrying C-terminal FGF23 (cFGF23) to inhibit FGF23 signalling in a mouse model for X-linked hypophosphataemic rickets (hyp-duk mice). They were able to show that a single injection of AAV cFGF23 rescued the hyp-duk phenotype.

X-linked hypophosphataemic rickets (XLH) is the most common form of rickets associated with elevated FGF23. Mutations in the *PHEX*-gene, encoding a metalloprotease, lead to high circulating FGF23 concentrations resulting in increased renal phosphate waste and impaired $1,25(OH)_2$-vitamin D production. FGF23 acts by binding to the FGF23 receptor and its co-receptor Klotho. cFGF23 competes for the binding to the FGF23-receptor/Klotho-complex blocking native FGF23 action.

This paper, using a mouse model for XLH (hyp-duk mice), describes a series of comprehensive experiments that are presented and analysed in detail. The experiments showed that a single injection of AAV cFGF23 resulted in efficient liver targeting. Only those animals that had received an AAV vector expressing cFGF23co-albumin showed increased blood phosphate levels, suggesting that cFGF23 alone is unstable. One injection of AAV cFGF23 restored the expression of the *Npt2a* transporter mRNA in the kidney. However, the normalization of blood phosphate levels was only transitory: after 3 months the blood phosphate level of treated mice was similar to controls. This could be explained either by phosphate reallocation to bone or by increased expression of *Nat2b* in the kidney. Interestingly, in contrast to treatment with the monoclonal human FGF23 antibody Burosumab, treatment with AAV cFGF23 did not affect the expression of the key enzymes involved in vitamin D metabolism nor did it affect circulating $1,25(OH)_2$-vitamin D levels. However, blood calcium rose to normal values in treated animals. Finally, the osteoarticular phenotype and partially bone length were restored in treated mice. Consequently, linear growth improved significantly in treated animals.

This study adds to present knowledge the proof of concept of the efficacy of liver-targeted gene therapy in a mouse model for XLH. Furthermore, in this XLH mouse model FGF23 regulation of vitamin D activation was unaffected. Nevertheless, blood calcium levels normalized in treated mice, suggesting a selective effect of cFGF23 on calcium regulation. Further work will be necessary to elucidate the molecular mechanism for this effect.

Genetics

14.15. Whole-genome risk prediction of common diseases in human preimplantation embryos

Akash Kumar, Kate Im, Milena Banjevic, Pauline C Ng, Tate Tunstall, Geronimo Garcia, Luisa Galhardo, Jiayi Sun, Oren N Schaedel, Brynn Levy, Donna Hongo, Dusan Kijacic, Michelle Kiehl, Nam D Tran, Peter C Klatsky, Matthew Rabinowitz
Nat Med. 2022 Mar;28(3):513–516.
doi: 10.1038/s41591-022-01735-0. Epub 2022 Mar 21.

Brief Summary: Currently, preimplantation genetic testing (PGT) is used to avoid specific rare Mendelian disorders before *in-vitro* fertilization (IVF). However, evidence is limited on the potential use of a polygenic risk score (PRS) that combines thousands of genetic variants as a predictor for common conditions, such as heart disease.

In this preclinical study, the authors sequenced the genomes of 10 couples and genotyped their 110 embryos in order to predict the inherited genome sequence of an embryo. They computed separate polygenic risk predictors for 12 common health conditions (e.g. autoimmune diseases) and compared the constructed genome and PRS to results obtained from a tissue sample of the corresponding newborn.

This paper demonstrates the accuracy of this whole-genome reconstruction (WGR) strategy, with over 95% for genotype prediction, which enables a confident polygenic risk scoring for the diseases evaluated. Furthermore, they showed the value of combining predictive information from common genetic variants (PRS) and rare variants, such as pathogenic variants in *BRCA1*. Limitations are still present – current available data means this approach is mostly relevant for European ancestry individuals, and ethical issues will need to be addressed. However, the approach seems promising for couples with high susceptibility to common genetic diseases who are undergoing IVF.

14.16. The mutational landscape of human somatic and germline cells

Luiza Moore, Alex Cagan, Tim H H Coorens, Matthew D C Neville, Rashesh Sanghvi, Mathijs A Sanders, Thomas R W Oliver, Daniel Leongamornlert, Peter Ellis, Ayesha Noorani, Thomas J Mitchell, Timothy M Butler, Yvette Hooks, Anne Y Warren, Mette Jorgensen, Kevin J Dawson, Andrew Menzies, Laura O'Neill, Calli Latimer, Mabel Teng, Ruben van Boxtel, Christine A Iacobuzio-Donahue, Inigo Martincorena, Rakesh Heer, Peter J Campbell, Rebecca C Fitzgerald, Michael R Stratton, Raheleh Rahbari

Nature. 2021 Sep;597(7876):381–386.
doi: 10.1038/s41586-021-03822-7.

Brief Summary: This study investigated the mutational background of somatic cells and rates of mutation in 29 distinct anatomical structures and compared these with the male germline from the same donor. The rate of mutation was lowest in spermatogonia.

Characterization of mutations that occur in human cells during the lifetime of an individual is challenging due to the differences between cell clones. Studies on somatic cell types have shown differences between clonal structures, rate of mutations and the effect of driver mutations on growth advantage. On the other side, trio analyses have demonstrated that ∼80% of inherited germline mutations arise in the paternal germline.

Mutation burden varies between the different cell types, most likely due to differences in mutation rates. The lowest mutation burden and rate was found in seminiferous tubules, predominantly composed of germline cells, indicating that the low germline mutation rate is an intrinsic feature of the male germline compared with the soma and not the result of sperm selection during development. Although the mechanisms producing mutagenesis seem to be the same for both germline and soma, the germline somehow is able to limit the mutation rate, which seems to be a beneficial strategy for reproduction.

Reviews

14.17. Prevention of atherosclerosis from childhood

Olli Raitakari, Katja Pahkala, Costan G Magnussen

Nat Rev Cardiol. 2022, 19(8):543-554.
DOI: 10.1038/s41569-021-00647-9

This review discusses the role of apo-lipoprotein B (apoB)-containing lipoproteins (commonly estimated by LDL cholesterol) in the process of atherosclerosis throughout the life-course. The authors comprehensively describe the development of atherosclerosis according to the response-to-retention hypothesis, and they present data from large-scale, intensive lifestyle intervention studies including children and adults showing moderate improvements in LDL cholesterol levels. However, substantial reductions in LDL cholesterol levels and a likely subsequent reduction in cardiovascular disease risk may only be achievable by wide use of pharmaceutical treatments, particularly statins.

The authors address the current dilemma when to use statins for primordial, and primary cardiovascular disease prevention in otherwise healthy children. Knowledge gaps include evidence on long-term safety of pharmaceutical therapies, data on effects from dietary and lifestyle interventions begun in childhood on adult cardiovascular morbidity and mortality, and missing data on possible crucial ages when risk factors have a particularly strong effect. Another key issue is that there is no consensus among the guidelines on screening strategies for dyslipidaemia in childhood.

The authors conclude that, in theory, cardiovascular diseases could be eradicated if the development of atherosclerosis could be prevented by sustaining healthy serum LDL cholesterol levels from childhood to adulthood. Consequently, future studies should aim at providing the evidence required to reach a consensus on strategies to identify and manage individuals at high risk of atherosclerosis, as the means to prevent this are seemingly present.

14.18. Endocrine manifestations and new developments in mitochondrial disease

Yi Shiau Ng, Albert Zishen Lim, Grigorios Panagiotou, Doug M Turnbull, Mark Walker
Endocr Rev. 2021 Oct 13;bnab036.
PMID: 34644386
DOI: 10.1210/endrev/bnab036

Brief Summary: This comprehensive review summarizes the current knowledge on the spectrum of endocrine disorders associated with mitochondrial disease. It provides essential information on the basics and genetics of mitochondrial diseases and details the clinical characteristics, focusing on endocrine and important non-endocrine features. It also provides guidelines/algorithms for diagnosis and management, e.g. for diabetes care and reproductive options.

30 years ago, maternal-inherited diabetes mellitus was first linked to mitochondrial DNA (mtDNA) pathogenic variants in 2 independent pedigrees, where it was found in combination with premature deafness across generations because of either a long mtDNA duplication/deletion (DOI: 10.1038/ng0892-368) or the m.3243A > G variant in the *MT-TL1* gene (DOI: 10.2337/diab.43.6.746). Meanwhile large cohort studies have shown that endocrine disorders, such as diabetes mellitus, adrenal and gonadal insufficiency, and hypoparathyroidism, may be prevalent and may precede other classic, clinical manifestations of mitochondrial disorders. On the other hand, in those affected patients with severe, complex other organ system failure (e.g. central or peripheral nervous system (prevalent in 80%), cardiac or kidney), the endocrine effects might be overlooked. Thus, as an endocrinologist it is important to recognize the "red flags" that hint at a mitochondrial origin to endocrine disorders, and to provide expertise on the optimal multi-disciplinary care to individual patients with often multiple organ failure.

Fortunately, the genetic revolution seen with NGS techniques has immensely improved the diagnostic yield of mitochondrial disease. Mitochondrial disorders occur in about 1:5000 of the general population with pathogenic variants in nuclear genes more prominent in childhood-onset forms (80%), and mtDNA variants more common in adult-onset cases. Overall, endocrine manifestations often relate to specific subtypes (genes) of mitochondrial disease and defects of oxidative phosphorylation (OXPHOS). But importantly, mitochondrial DNA genetics differs fundamentally from nuclear DNA genetics. In brief: a) maternal inheritance only, b) cells contain multiple (hundreds to thousands) of mtDNA copies, c) pathogenic mtDNA variants can affect all copies (homoplasmy) or only some (heteroplasmy), thus heteroplasmic defects are mostly functionally recessive and require a threshold to become disease-causing, d) heteroplasmy may be tissue specific and change over time. Thus testing for pathogenic mtDNA variants has to be done with great care using blood, urine sediment or tissue (i.e. muscle) and techniques, such as either targeted pyrosequencing or amplification of mtDNA followed by NGS, which allow sensitive detection of single nucleotide changes and large-scale deletions, as well as quantification of mutant load.

Reproductive options to avoid mitochondrial disease include preimplantation and prenatal testing as well as mitochondrial replacement therapy from healthy donors, but the latter is still an experimental technique in embryos and not in routine use.

14.19. Cytoplasmic DNA: sources, sensing, and role in aging and disease

Karl N Miller, Stella G Victorelli, Hanna Salmonowicz, Nirmalya Dasgupta, Tianhui Liu, João F Passos, Peter D Adams
Cell. 2021 Oct 28;184(22):5506–5526.
doi: 10.1016/j.cell.2021.09.034

Brief Summary: The authors review the function and the underlying molecular mechanisms of 4 major species of endogenous cytoplasmic DNA (cytoDNA) in the development of chronic ageing-associated diseases.

In the presence of foreign DNA (e.g. viral infections), innate immunity acts as a defence mechanism that produces cytokines and chemokines to activate the inflammatory cell response. Key cellular sensors that trigger

innate immune system signalling pathways and are responsible of recognizing external molecular patterns, including nucleic acids, are well known. For example, STING, a molecule that stimulates interferons, is important for the recognition of cytoDNA and enhances the expression of genes involved in the innate immune system that react to several DNA pathogens. In the absence of a pathogen, the presence of DNA in the cytoplasm is interpreted as toxic and the defences of the immune system are activated. This inflammatory reaction, called 'sterile inflammation', has been associated with cancer, ageing and neurodegenerative diseases, which are accompanied by a chronic level inflammation.

The role of endogenous cytoDNA in the activation of the innate immune system is well-known, but their contribution to the development of chronic diseases remains unclear. Studies of the function and the biological formation of the different cytoDNAs revealed similar potential mechanisms, such as the loss of nuclear membrane integrity for cytosolic accumulation of micronuclei and retrotransposons. It is suggested that further understanding of these processes might contribute to novel therapeutic opportunities in the treatment of related human disease.

15. Editors' Choice

Ken K Ong, Christa E Flück

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Obesity

15.1. Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes

Frias JP, Davies MJ, Rosenstock J, Perez Manghi FC, Fernandez Lando L, Bergman BK, Liu B, Cui X, Brown K, SURPASS-2 Investigators

N Engl J Med. 2021;385(6):503-15.

DOI: 10.1056/NEJMoa2107519.

PubMed ID: 34170647

Brief summary: this randomized control trial in 1879 adults with type 2 diabetes compared effects of once-weekly Tirzepatide (5, 10 or 15 mg) versus once-weekly Semaglutide (1 mg) on glycemic control. After 40 weeks, all doses of Tirzepatide were superior to Semaglutide on lowering HbA1c.

Glucagon-like peptide-1 (GLP-1) receptor agonists, Liraglutide (given once-daily) and Semaglutide (once-weekly), are increasingly used in adult patients with obesity and impaired glucose tolerance or type 2 diabetes, following consistent trial evidence regarding their effectiveness to reduce body weight and lower glucose levels. Liraglutide is licensed for use in children aged 10 years and older with type 2 diabetes and we are eagerly looking forward to its wider licensing and funding approvals, as well as similar approvals for Semaglutide. However there are other gut derived peptides beyond GLP-1, which may have similar potent effects on body weight and glucose metabolism.

Tirzepatide is a 'dual agonist', which combines actions on GLP-1 receptors and also on glucose-dependent insulinotropic polypeptide (GIP) receptors. A major pharmacokinetic advantage is its once weekly administration, although like Semaglutide it is given by subcutaneous injection. The current findings are impressive, showing that the dual agonist Tirzepatide at all doses tested is superior to the single agonist Semaglutide in controlling glycemic levels in type 2 diabetes. Tirzepatide was subsequently approved for this indication by the FDA in May 2022.

However as well as its superior effects on HbA1c, in this trial Tirzepatide was also more effective than Semaglutide in reducing body weight, again at all doses. Further data released in April 2022, confirmed the remarkable effectiveness of Tirzepatide on body weight, which was on average 24 kg lower on the 15 mg dose after 72 weeks (1). We look forward to seeing this evidence in publication, as well as further evidence on the effects of both Tirzepatide and Semaglutide on body weight and HbA1c in children.

15.2. Postprandial glycaemic dips predict appetite and energy intake in healthy individuals

Wyatt P, Berry SE, Finlayson G, O'Driscoll R, Hadjigeorgiou G, Drew DA, Khatib HA, Nguyen LH, Linenberg I, Chan AT, Spector TD, Franks PW, Wolf J, Blundell J, Valdes AM

Nat Metab. 2021;3(4):523-9.
DOI: 10.1038/s42255-021-00383-x.
PubMed ID: 33846643

Brief summary: this study of around 1000 adults administered standard breakfast meals and showed wide variability and continuously monitored glucose levels up to 3 h afterwards. Notably, those individuals with lower glucose levels at 2 to 3 h after meals reported higher levels of appetite and consumed hundreds more calories each day.

It is increasingly recognized that, not only patients with diabetes, but also healthy individuals vary widely in their glycemic responses to food and other lifestyle factors. This study from Kings College London efficiently collected data through continuous glucose monitors and the ZOE app (which has been made famous by its collection of data on COVID-19). They also sent out standardized breakfasts (muffins!) hence collecting data on over 8000 breakfasts.

While previous studies have focused on peak glucose levels following oral glucose challenge or standard meals, the current study recognized that many people have significant dips in glucose levels 2 to 3 h after meals. They observed that these individuals reported higher levels of hunger and consumed 75 calories more by 3 to 4 hours after breakfast, and over 300 calories more across the whole day. These findings were confirmed in a US validation cohort.

The underlying mechanisms are unclear and could involve brain sensing of changes in glucose, central actions of insulin, and interactions with the gut microbiome. However, regardless of the mechanisms, the authors are optimistic that awareness of our individual responses to food may help us identify those foods that lead to more stable changes in our personal glucose profiles and hence give us greater control over our appetites and weight gain.

15.3. Obesity-associated GNAS mutations and the melanocortin pathway

Mendes de Oliveira E, Keogh JM, Talbot F, Henning E, Ahmed R, Perdikari A, Bounds R, Wasiluk N, Ayinampudi V, Barroso I, Mokrosinski J, Jyothish D, Lim S, Gupta S, Kershaw M, Matei C, Partha P, Randell T, McAulay A, Wilson LC, Cheetham T, Crowne EC, Clayton P, Farooqi IS
N Engl J Med. 2021;385(17):1581-92.
DOI: 10.1056/NEJMoa2103329.
PubMed ID: 34614324

Brief Summary: The authors performed whole exome sequencing in 2548 children with severe obesity and identified 22 *GNAS* mutation carriers, almost all of which disrupted melanocortin 4 receptor (MC4R) signaling.

The gene *GNAS* encodes the stimulatory G-protein alpha subunit protein, which is a key component of many hormone receptors (GPCRs). Deleterious GNAS mutations are well known to cause Albright's hereditary osteodystrophy, characterised by short stature and skeletal abnormalities. Furthermore, as it is a paternally imprinted (silenced) gene, maternally-inherited GNAS mutations also cause resistance to parathyroid hormone (pseudohypoparathyroidism) as well as resistance to diverse other hormones.

A characteristic effect of pseudohypoparathyroidism on early onset obesity associated with hyperphagia has long been recognised (1), however the underlying mechanism was unknown. Here, the authors show that 22 (of 2548) children with severe obesity carried deleterious mutations in *GNAS*, most of which disrupted signaling of MC4R, the well-described hypothalamic regulator of appetite and weight gain. This confirms *GNAS* as a cause for monogenic obesity associated with hyperphagia. The authors comment that severely obese patients with *GNAS* mutations should respond to the MC4R agonist, setmelanotide.

In addition, the wide involvement of the stimulatory G-protein alpha subunit across diverse hormone receptors means that affected children have a complex endocrine phenotype. 6 of the 11 patients who were age 12 to 18 years had evidence of reduced growth (mean standard-deviation score for height, −0.90), and *in vitro* their mutations disrupted growth hormone-releasing hormone receptor signaling. Furthermore, *GNAS* mutations that impaired thyrotropin receptor signaling were associated with developmental delay and high TSH levels (mean [±S.D.], 8.4±4.7 mIU/l).

By contrast, although most identified *GNAS* mutations impaired PTH receptor signaling, only one patient had subcutaneous ossifications, and 10 patients showed mild brachydactyly, two characteristics of Albright's hereditary osteodystrophy (OMIM 103580).

Reference

1. Ong KK, Amin R, Dunger DB. Pseudohypoparathyroidism–another monogenic obesity syndrome. *Clin Endocrinol (Oxf)*. 2000 Mar;52(3):389–91. PMID: 10718839.

Diabetes

15.4. Four groups of type 2 diabetes contribute to the etiological and clinical heterogeneity in newly diagnosed individuals: An IMI DIRECT study

Wesolowska-Andersen A, Brorsson CA, Bizzotto R, Mari A, Tura A, Koivula R, Mahajan A, Vinuela A, Tajes JF, Sharma S, Haid M, Prehn C, Artati A, Hong MG, Musholt PB, Kurbasic A, De Masi F, Tsirigos K, Pedersen HK, Gudmundsdottir V, Thomas CE, Banasik K, Jennison C, Jones A, Kennedy G, Bell J, Thomas L, Frost G, Thomsen H, Allin K, Hansen TH, Vestergaard H, Hansen T, Rutters F, Elders P, t'Hart L, Bonnefond A, Canouil M, Brage S, Kokkola T, Heggie A, McEvoy D, Hattersley A, McDonald T, Teare H, Ridderstrale M, Walker M, Forgie I, Giordano GN, Froguel P, Pavo I, Ruetten H, Pedersen O, Dermitzakis E, Franks PW, Schwenk JM, Adamski J, Pearson E, McCarthy MI, Brunak S, Consortium ID

Cell Rep Med. 2022;3(1):100477.

DOI: 10.1016/j.xcrm.2021.100477.

PubMed ID: 35106505

Brief summary: To explore clinical heterogeneity, this study analyzed baseline visit data on 726 adults with newly diagnosed Type 2 diabetes (T2D) adults and identified in 4 distinct profiles (clusters of phenotypes), which predicted differences in subsequent disease progression and anti-diabetic treatments.

It is increasingly recognised that T2D is not a homogenous condition. Separate to this study, recent data show that adolescents with T2D display a more rapidly progressing form of disease associated with need for insulin therapy and high incidence of microvascular complications (see Paper 12.3 in this Yearbook).

This study focussed on T2D in adults, who had at study baseline a mean age of 62 years, mean BMI 30.4 kg/m^2, and all were on lifestyle and/or metformin treatment only. They were characterised at baseline in great depth, by a wide range of anthropometry, hormones, lipids, and a frequently-sampled mixed-meal tolerance test. Diabetes progression was assessed by change in HbA1c over the 36 months follow-up. A soft-clustering statistical method was used to sub-group patients based on 32 clinical variables. Four different profiles ('archetypes') were identified. Patients with a baseline archetype characterised by obesity, insulin resistance, dyslipidemia, and impaired beta cell function ($N=45$ individuals, 6% of total) showed the fastest disease progression. Other archetypes included: A) low BMI, older age, high insulin sensitivity, and high cholesterol ($N=103$; slowest HbA1c progression); B) high BMI, but insulin sensitive and favorable lipid profiles ($N=22$); C) high BMI, insulin resistance ($N=84$).

A limitation is that only 35% of patients could be confidently categorised to one archetype, and the majority displayed phenotypes with moderate contributions from two or more archetypes, possibly due to multiple etiological processes. Furthermore, over time there was mixed stability in archetypes. Hence, the study tests a promising idea to map the wide variations in disease presentations to different aetiological processes and inform personalized treatments. However, clustering using even more traits (e.g. omics, genetics, continuous glucose monitoring) may be needed to achieve those aims.

15.5. Trends in prevalence of type 1 and type 2 diabetes in children and adolescents in the US, 2001–2017

Lawrence JM, Divers J, Isom S, Saydah S, Imperatore G, Pihoker C, Marcovina SM, Mayer-Davis EJ, Hamman RF, Dolan L, Dabelea D, Pettitt DJ, Liese AD, Group SfDiYS

JAMA. 2021;326(8):717-27.
DOI: 10.1001/jama.2021.11165.
PubMed ID: 34427600

Brief summary: This large repeated cross-sectional study identified all individuals aged <20 years with physician-diagnosed diabetes in 6 areas in the US in 2001, 2009, and 2017. Over these 16 years, the prevalence of Type 1 diabetes (T1D) increased by 45% (95% CI, 40%–50%) and the prevalence of Type 2 diabetes (T2D) almost doubled (relative increase 95.3%; 95% CI, 77.0%–115.4%).

This comprehensive study quantifies the alarming rates of rise in both T1D and T2D. Data were collected from clinical centers in each area, and each survey year covered a mean estimated 3.47 million youths aged <20 years from 4 geographic areas, 1 health plan, and select American Indian reservations. The rise in T1D was more marked between 2001–2009 (+3.4% per year) than 2009–2017 (+1.4% per year), whereas the rise in T2D accelerated from 2001–2009 (+3.7% per year) to 2009–2017 (+4.8% per year). Furthermore, Black and Hispanic youths showed the greatest absolute increases in the prevalences of both T1D and T2D.

The authors hypothesize that secular changes in obesity and ethnic composition of the population contributed to the rise in T2D. They were puzzled by the rise in T1D, which they conjectured is due to infectious and mucosal exposures in the first 2 years. However, Paper 10.4 in this Yearbook identifies that not only T2D but also T1D risk is enhanced by childhood overweight and obesity (1).

These data are accompanied this year by similar highly concerning statistics showing that the incidence of Gestational Diabetes (GDM) rose by one third over 8 years, between 2011 to 2019 in the US (2). Together, these findings show that we are losing the battle not only with obesity, but also with its major glycaemic metabolic consequences.

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2. Shah NS, *et al*. Trends in Gestational Diabetes at First Live Birth by Race and Ethnicity in the US, 2011–2019. *JAMA*. 2021;326(7):660–669. doi:10.1001/jama.2021.7217.

New Hormones

15.6. A hormone complex of FABP4 and nucleoside kinases regulates islet function

Prentice KJ, Saksi J, Robertson LT, Lee GY, Inouye KE, Eguchi K, Lee A, Cakici O, Otterbeck E, Cedillo P, Achenbach P, Ziegler AG, Calay ES, Engin F, Hotamisligil GS

Nature. 2021;600(7890):720-6.
DOI: 10.1038/s41586-021-04137-3.
PubMed ID: 34880500

Brief summary: This mouse study identifies FABP4 (‘Fabkin’) as a new fat-derived hormone that regulates insulin-producing beta cells in the pancreas.

We have learnt over the recent years that body fat is not simply a passive energy storage tissue, but instead secretes a number of active peptides that influence appetite regulation, insulin sensitivity and inflammatory pathways. These authors now identify a new adipocyte-derived hormone, which they call ‘Fabkin’, which is released on lipolysis and acts to stimulate pancreatic islet cell function.

There are several unique characteristics of this new hormone. The authors claim that Fabkin is the only known hormone that is released from adipose tissue by lipolysis. Unlike traditional hormones which typically comprise a single molecule and act on a single receptor, Fabkin comprises a functional multi-protein complex, including fatty acid binding protein 4 (FABP4), adenosine kinase (ADK) and nucleoside diphosphate kinase (NDPK). Furthermore, it establishes a novel adipocyte-beta cell endocrine axis.

In humans, FABP4 is increased in type 2 diabetes (T2D) and correlates with body–mass index (BMI), and the authors also showed that circulating FABP4 is ∼1.6-fold higher in patients with new-onset T1D compared with

non-diabetic individuals. In mice, blocking Fabkin using antibody-mediated targeting of the hormone complex improved metabolic outcomes, enhanced beta-cell function and preserved beta-cell integrity in mouse models of type 1 and type 2 diabetes. Beyond the beta cell, Fabkin showed a diverse activity profile, including on inflammation, insulin resistance and cardiac pathophysiology. Is this too good to be true, or will Fabkin be a future therapeutic target for obesity-related metabolic diseases?

15.7. A nutrient-specific gut hormone arbitrates between courtship and feeding

Lin HH, Kuang MC, Hossain I, Xuan Y, Beebe L, Shepherd AK, Rolandi M, Wang JW
Nature. 2022;602(7898):632-8.
DOI: 10.1038/s41586-022-04408-7.
PubMed ID: 35140404

Brief summary: This study in the fruit-fly, Drosophila melanogaster, identified a novel gut-derived, nutrient-specific neuropeptide hormone, 'Diuretic hormone 31' (Dh31), which triggers the insects to switch their behaviour from feeding to courtship and reproduction.

Romantic dinners are ingrained in human culture as an essential component of courtship, love and relationships. However, I think few of us suspected that this behaviour is based on a direct physiological connection!

The authors found that protein-rich foods triggered the release of Dh31 from enteroendocrine cells in the gastrointestinal tract. Unsurprisingly, starved males prioritized feeding behaviours above courtship. However, remarkably after consumption of protein-rich food this behaviour rapidly changed within only a few minutes. They used functional imaging and optogenetic stimulation to show that circulating Dh31 rapidly excites brain neurons that express the Dh31 receptor (Dh31R). They identified two distinct populations of Dh31R+ neurons in the brain; stimulation of one population inhibited feeding through allatostatin-C, and the other population triggered courtship through corazonin.

It seems amazing that a single molecule can have such a marked influence on behaviour. Courtship in these flies includes a complex array of behaviours, including performing a courtship song to entice females, through to reproductive activity. The authors draw parallels with other hormones that alter our behaviours: orexin on the switch from awake to sleep, leptin on the switch from hungry to feeling full, and possibly also prolactin on promoting warm relational connections with others.

Assorted Conditions

15.8. Serum testosterone levels in 3-month-old boys predict their semen quality as young adults

Scheutz Henriksen L, Holm Petersen J, Skakkebaek NE, Jorgensen N, Virtanen HE, Priskorn L, Juul A, Toppari J, Main KM
J Clin Endocrinol Metab. 2022;107(7):1965-75.
DOI: 10.1210/clinem/dgac173.
PubMed ID: 35323957

Brief summary: This population-based birth cohort study related infancy serum testosterone concentrations at age 3 months to parameters of reproductive function at age 18 to 20 years in 259 males. Serum testosterone in infancy predicted adult total sperm counts, and other reproductive hormones and genital measures showed good correlations between infancy and adulthood.

The 'minipuberty' of infancy is well recognised by Paediatric Endocrinologists as a window of endogenous sex hormone activity, during which we can very usefully investigate patients with suspected hypothalamo-pituitary or gonadal disorders. However, beyond our professional community, the existence of this phenomenon is surprising and, even to us, the physiological role of this process is largely unknown.

These authors are leading researchers in reproductive biology and clinical reproductive disorders, and they set up the Copenhagen Mother-Child cohort to first characterise the physiological changes of infant minipuberty. This long-term follow-up of the cohort provides unique and powerful data showing the remarkable correlations between infancy and adult reproductive function. Median (IQR) total sperm counts ranged from 84 (54-138) million spermatozoa for boys in with the lowest infancy testosterone tertile, to 193 (56-287) million spermatozoa in the highest tertile. Infant-adult correlations in sex hormone levels were highest for FSH, and lower but significant for inhibin B, SHBG, penile length, and testis volume. Another recent paper by this group showed that male minipuberty shows a surprising temporal dissociation of Leydig and Sertoli cell activity, which peak at ages 1 month and 4-5 months, respectively (1). Normal reference data are provided for reproductive hormones concentrations in male infants.

The current findings suggest that a boy's reproductive set-point is apparent shortly after birth and persists to adult life. While this provides a valuable early life window into our patients' later reproductive potential, an important question is whether these links reflect inherent (e.g. genetic) set-points, or whether later reproductive function might also be programmed by disruption and/or therapeutic early hormone replacement interventions?

Reference

1. Busch AS, *et al*. Dynamic Changes of Reproductive Hormones in Male Minipuberty: Temporal Dissociation of Leydig and Sertoli Cell Activity. The Journal of Clinical Endocrinology & Metabolism, Volume 107, Issue 6, June 2022, Pages 1560–1568, https://doi.org/10.1210/clinem/dgac115.

15.9. Genetic insights into biological mechanisms governing human ovarian ageing

Ruth KS, Day FR, Hussain J, Martinez-Marchal A, Aiken CE, Azad A, Thompson DJ, *et al*.
Nature. 2021;596(7872):393-7.
DOI: 10.1038/s41586-021-03779-7.
PubMed ID: 34349265

Brief summary: This study analysed genome-wide association array (GWAS) data on ~200 000 women of European ancestry to identify 290 separate genetic signals associated with normal variation in age at natural menopause (ANM). Experimental alterations of key identified genes in mouse models confirmed their impacts on ovarian ageing, but also identified impacts across the life-course that shape fetal generation of the ovarian reserve as well as its rate of postnatal depletion.

Clinicians and scientists have long considered menopause to be a marker of biological aging, with little obvious relationship with childhood reproductive development or pubertal traits. Indeed this concept was reinforced by previous GWAS findings that various DNA damage response (DDR) processes contribute substantially to the genetic predisposition to earlier or later ANM. Apart from rare genetic causes of premature ovarian failure (Turner syndrome) or severe external perturbations (radiotherapy and chemotherapy), Paediatricians rarely consider the links between common childhood presentations and later menopause.

These researchers showed directionally-opposing effects of different DDR pathways on ANM. Disruption of DDR genes that repair damaged DNA led to a smaller oocyte pool, faster decline in oocyte numbers and earlier ANM (e.g. *Chek1*). By contrast, disruption of DDR genes (e.g. *Chek2*) that detect DNA damage and trigger cell apoptosis (i.e. trigger cell death instead of damage repair) led to more oocytes numbers and later ANM (albeit the oocyte retain DNA damage). Hence, female fertility in mice could be prolonged by *Chek2* deletion or *Chek1* overexpression. A key surprising observation was that *Chek2* deletion and *Chek1* overexpression increased ovarian oocyte pool size in infancy, as well as in later life.

These findings show that the same biological processes that protect and respond to cell damage in later life also have fundamental roles during early life development, when rates of cell replication and differentiation are high and cells are prone to external stressors, such as oxidative damage. Disruption of these processes may have impacts on reproductive development and functioning across the life-course.

15.10. Metabolomic profiling reveals extensive adrenal suppression due to inhaled corticosteroid therapy in asthma

Kachroo P, Stewart ID, Kelly RS, Stav M, Mendez K, Dahlin A, Soeteman DI, Chu SH, Huang M, Cote M, Knilhtila HM, Lee-Sarwar K, McGeachie M, Wang A, Wu AC, Virkud Y, Zhang P, Wareham NJ, Karlson EW, Wheelock CE, Clish C, Weiss ST, Langenberg C, Lasky-Su JA

Nat Med. 2022;28(4):814-22.
DOI: 10.1038/s41591-022-01714-5.
PubMed ID: 35314841

Brief summary: This study performed large-scale metabolomic profiling across 14 000 adults from 4 cohorts and identified 17 steroid metabolites whose levels were reduced in individuals with prevalent asthma. The largest reductions were associated with inhaled corticosteroid (ICS) treatment, and these were validated in samples from a clinical trial of low-dose ICS.

Even our non-endocrine colleagues and many patients are aware of the harmful effects of high dose oral corticosteroids (CS) on adrenal suppression. However, we have long considered ICS to be a relatively safe option for asthma, except at occasionally used very high doses. Here, the authors used the power of large-scale metabolomic profiling of random plasma samples to generate data on 973 metabolites. Only a minority of the metabolites tested were annotated to steroid metabolism (sub-)pathways, but most of these showed lower levels in those taking ICS. In a clinical cohort, they found that also circulating cortisol levels were lower in patients using ICS, with the largest difference seen in early morning samples. Furthermore, patients on ICS showed more symptoms of fatigue and anaemia, despite no difference in body weight.

Hence, adrenal suppression in patients with asthma treated with ICS might be more extensive than previously recognized. Widespread screening of adrenal function by dynamic testing of all children and adults taking ICS is clearly unfeasible. Instead, such patients should at least be made aware of the possible related symptoms of adrenal suppression, such as fatigue, headache, abdominal pain, vomiting and psychiatric symptoms. Furthermore, prescribing physicians and patients with asthma should be encouraged to use ICS at the lowest effective dose, to mitigate its systemic absorption and adverse effects. And, if in doubt, to test and treat patients taking ICS for subclinical adrenal insufficiency.

15.11. Evidence that ageing yields improvements as well as declines across attention and executive functions

Verissimo J, Verhaeghen P, Goldman N, Weinstein M, Ullman MT

Nat Hum Behav. 2022;6(1):97-110.
DOI: 10.1038/s41562-021-01169-7.
PubMed ID: 34413509

Brief summary: This cross-sectional study of adults ($N = 702$) aged 58–98 measured cognitive abilities across a range of domains (alerting, orienting and executive inhibitory networks) by use of a computer-based Attention Network Test. In non-linear regression models, while efficiency of the alerting network declined with age, orienting and executive inhibitory efficiency improved with age up to the mid-to-late 70s.

This topic is no doubt of interest to many of our 'more mature' readers (and editors!). We generally assume, and many scientists believe, that cognitive functions, such as attention, executive function, and reasoning skills, decline with age. This study challenges that belief. In a large sample spanning a wide age range, they find that certain cognitive functions improve with age, even to late 70s. A decline was seen for *alerting efficiency*, for example the heightened preparedness when you approach a junction while driving a car. Conversely, age-related improvements were seen in *orienting*, which occurs when you shift your attention to an unexpected movement such as a pedestrian or cyclist, and also in *executive inhibitory efficiency,* which allows you to inhibit distractions, for example due to your noisy passengers or interesting sights next to the road.

An exciting implication of the findings relates to the use of 'brain training' exercises. It seems most unlikely that the improvements are due to anatomical neural development at such advanced ages. Instead, the authors suggest

that some abilities improve because adults practice these skills throughout their life. The authors comment that it may certainly be possible to enhance executive inhibitory function with training and practice. Beyond the traits measured here, it is well recognised that some cognitive functions decline with ageing, especially our memory and ability to learn new skills. However, cognitive skills that improve with practice are usually well preserved, such as understanding and use of language.

15.12. One statistical analysis must not rule them all

Wagenmakers EJ, Sarafoglou A, Aczel B
Nature. 2022;605(7910):423-5.
DOI: 10.1038/d41586-022-01332-8.
PubMed ID: 35581494

Brief summary: This *Nature* comment was prompted by the very wide range of calculated values for the COVID-19 infections 'reproduction number' *R*, produced by different modelling teams despite access to the same datasets on UK's emerging '2nd wave' in October 2020. Values of R ranged from 115 new infected individuals (infected from 100 individuals infected at baseline) with a lower confidence limit of 96 (i.e. slightly declining infections) to 166 with an upper limit of 182 (i.e. almost doubling). They argue that no one answer is correct, rather the range of values indicates true uncertainty in the data.

This concept may seem bizarre to you – if so, I strongly encourage you to read this eloquent and well-illustrated article in full. While rarely (I hope) some published statistics might be due to use of invalid analyses or even deliberate 'massaging' of the data, these authors show that a range of valid statistical approaches exist to answer the same question with the same datasets. Choice, classification, and type of adjustment for potential confounders is one simple example of the possible differences. Another may be the decision to use non-parametric tests for skewed data, or to transform values to allow parametric tests. Indeed, in other 'multi-analyst' projects, independent statisticians rarely use the same procedure.

In October 2020, the UK was concerned about the possible re-emergence of COVID-19 (the '2nd wave'). The Scientific Pandemic Influenza Group on Modelling asked 9 teams to calculate the reproduction number R for COVID-19 infections. As well as differing modelling approaches, another source of variability between teams was likely their decision to prioritise the abundance of data available (on deaths, hospital admissions, testing rates). No two teams produced the same estimates.

The authors describe that such multi-team approaches are commonly used in the fields of high-energy physics, astronomy and climate modelling. They argue for the adoption of similar working in other fields where we place great importance on a single result, such as medicine, psychology, materials science, ecology, etc...

15.13. The effects of remote work on collaboration among information workers

Yang L, Holtz D, Jaffe S, Suri S, Sinha S, Weston J, Joyce C, Shah N, Sherman K, Hecht B, Teevan J
Nat Hum Behav. 2022;6(1):43-54.
DOI: 10.1038/s41562-021-01196-4.
PubMed ID: 34504299

Brief summary: This study analysed a large database of emails, calendars, instant messages, video/audio calls and workweek hours routinely generated by 61 182 US Microsoft employees in the months before and after 4th March 2020, when the company mandated that all non-essential employees change to full-time working from home (WFH). Remote working led to more 'siloed' work groups, fewer phone calls but more e-mails.

While most of our clinical duties and laboratory-based research requires on-site working, we learnt during the COVID-19 pandemic that many types of work can continue quite efficiently by WFH. My own experience in epidemiology research (KO) was that PhD students and post-doctoral scientists in my team continued to train and work very productively using a variety of online platforms to connect us while working across 6 different countries. However, some aspects of work suffered, particularly those that rely on spontaneous interactions, such as cross-team sharing of ideas and inter-personal sharing of advice, experiences and broader pastoral support.

This study formally documents and quantifies what many of us have come to realise about remote working. While remote working strengthened many interactions within workers' immediate teams, across the wider company the network of interactions became fragmented, making it harder for staff to gain and share new information across the network. Workers spent more time interacting with those linked by existing strong ties. However, the different business groups within Microsoft became less interconnected. While there are benefits for the efficient achievement of the defined short to medium-term goals, creative working and ideas that result from multi-disciplinary interactions suffer. The authors comment that when 2 people are connected by a strong tie, they more easily trust each other, cooperate and share information easily. By contrast, weak ties are more likely to provide access to new, non-redundant information.

Similar lessons likely apply to our interactions with those outside of our own institutions. So we do hope that you are able to enjoy the ESPE 2022 meeting in person and, through both formal and informal networking, you gain and share much new, non-redundant information, as well as new friends!

Basic Science & Genetics

15.14. Population-based penetrance of deleterious clinical variants

Forrest IS, Chaudhary K, Vy HMT, Petrazzini BO, Bafna S, Jordan DM, Rocheleau G, Loos RJF, Nadkarni GN, Cho JH, Do R
JAMA. 2022;327(4):350-9.
DOI: 10.1001/jama.2021.23686.
PubMed ID: 35076666

Brief summary: This study assessed the penetrance of pathogenic and loss-of-function clinical variants in 2 large population-based biobank studies. It found that the penetrance of pathogenic/loss-of-function variants was variable but generally low among such unselected populations.

We are increasingly using genetic tests to confirm the diagnosis in patients whom we suspect may have a (often rare) monogenic disease. Such tests use DNA sequencing of the patient to detect those who have pathogenic and loss-of-function clinical variants in known disease causing genes. However, as genetic sequencing is becoming more popular, for example as a screening test in ill patients or even as a predictive tool used by healthy individuals, it is becoming clear that the diagnostic value of a positive test result is much weaker than we are used to. This is because, in our usual clinical practice, we highly select patients for genetic tests based on their clinical presentation; e.g. we select patients with early onset T2D and positive family history for MODY (Maturity-Onset Diabetes in Youth) for gene sequencing – and all patients who return a positive genetic test are given the diagnosis of MODY. By contrast, if we perform the same test in 100 000 unselected adults, only 20–30% of those with 'pathogenic' mutations in *HNF1A* and *HNF4A* actually have diabetes (assessed by HbA1c testing and/or clinical records) (1).

The current study used the same approach across a wide range of genes for 197 diverse monogenic disorders in large biobank studies, the UK Biobank and the BioMe Biobank (Mount Sinai Health System in Manhattan, New York), who underwent whole exome sequencing. There was very wide variation in the penetrance of known pathogenic mutations. Even for well-established gene-disease pairs, the penetrance of pathogenic/loss-of-function variants varied enormously: in *LDLR* for familial hypercholesterolemia (penetrance 0%–100%); in *BRCA1*, *BRCA2*, and *PALB2* for breast cancer (0%–100%).

These consistent findings challenge our notion that pathogenic mutations for monogenic disease should segregate highly (almost perfectly) with disease status. Instead, the prior reasons for choosing a test are crucial for the interpretation of the result. Furthermore, the predictive utility of genomic testing as a screening tool in wider populations requires further consideration.

Reference

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15.15. Accurate prediction of protein structures and interactions using a three-track neural network

Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD, Millan C, Park H, Adams C, Glassman CR, DeGiovanni A, Pereira JH, Rodrigues AV, van Dijk AA, Ebrecht AC, Opperman DJ, Sagmeister T, Buhlheller C, Pavkov-Keller T, Rathinaswamy MK, Dalwadi U, Yip CK, Burke JE, Garcia KC, Grishin NV, Adams PD, Read RJ, Baker D

Science. 2021;373(6557):871-6.
DOI: 10.1126/science.abj8754
PubMed ID: 34282049

Brief summary: This study reveals a Deep Learning method, 'RoseTTA fold', based on DeepMind's Alphafold2 framework, to predict 3-dimensional protein structures from 1-dimensional sequence information and generate models of protein–protein complexes with high accuracy.

Previous Yearbook commentaries have described the application of machine learning ('artificial intelligence', AI) approaches to science and medicine questions, such as growth modelling and adult height prediction. Prediction of 3-dimensional protein structures is a challenge that is several orders of magnitude tougher, due to the endless possible amino-acid sequence compositions and interactions. It also offers important rewards. The authors used 150K known structures as a training set and illustrate the potential advances in understanding by application to MC4R ligand binding. These academic authors, from the University of Washington, Seattle, acknowledge that RoseTTA fold is not quite as accurate as Alphafold2, which had been announced in 2020 but kept private by its developer, DeepMind, an AI company owned by Google. A valuable achievement of the current paper was that it prompted DeepMind to publish and make publically available Alphafold2 on the same day (1). RoseTTA also has the advantage of predicting protein interactions and protein complexes.

These computer approaches now can solve in a few hours an important challenge that could typically take 1 year to achieve using traditional laborious laboratory techniques, such as X-ray crystallography. Knowledge of such structures can help to identify the key functional parts of a protein, can be used to model the structural and functional consequences of genetic mutations, and valuably predict the nature of protein–protein interactions, such as natural or synthetic ligand–receptor interactions, and also epitope–antibody interactions. Thus, such protein characterisation is fundamental for designing targets in the development of new drugs.

References

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15.16. 100,000 genomes pilot on rare-disease diagnosis in health care – preliminary report

The 100 000 Genomes Project Pilot Investigators

N Engl J Med. 2021;385(20):1868-80.
DOI: 10.1056/NEJMoa2035790.
PubMed ID: 34758253

Brief summary: This paper describes the pilot data for the UK 100 000 Genomes Project, including Whole Genome Sequencing (WGS) data on 4660 participants from 2183 families, and covering 161 disorders. They made a genetic diagnosis in 25% of probands; diagnoses were more likely for probands who presented with intellectual disability, hearing, or visual disorders (range 40% to 55%) and in cases who had WGS data on family trios (both their parents) or larger family pedigrees. Of the genetic diagnoses made, 25% had immediate implications for clinical decisions for the probands or their relatives.

The last year has seen a rapid increase in the numbers of publications of rare genetic variants associated with complex diseases, due to the recent availability of Whole Exome Sequence (WES) data in large-scale population-based studies. For example, WES data on over 650K people from the UK Biobank and FinnGen cohorts identified 975 associations between rare, protein-coding variants with 148 disease clusters (1).

The current paper leaps ahead to describe what will be the additional value of massively extending the scope of DNA sequences covered by moving from WES (which focusses on gene exons) to WGS. While it is expected that most disease-causing mutations would be located in/near gene exons, WES coverage of exons is imperfect and a notable finding is that 8% of the coding (amino acid changing) variants detected by the broader WGS approach had been missed on WES. Furthermore, 14% of rare diagnoses made by WGS involved noncoding, structural, or mitochondrial genome variants. This is an exciting era for human genetics, with the increasing availability of data on rare dysfunctional variants as well as large scale GWAS array data for common variants. This study shows that the use of WGS, providing more comprehensive genome coverage, will enable significant new insights into the genetic determinants of disease.

Reference

1. Sun BB, *et al*. Genetic associations of protein-coding variants in human disease. *Nature* Feb 2022. 603; 95–102.

15.17. Sex-specific genetic regulation of adipose mitochondria and metabolic syndrome by Ndufv2

Chella Krishnan K, Vergnes L, Acin-Perez R, Stiles L, Shum M, Ma L, Mouisel E, Pan C, Moore TM, Peterfy M, Romanoski CE, Reue K, Bjorkegren JLM, Laakso M, Liesa M, Lusis AJ
Nat Metab. 2021;3(11):1552-68.
DOI: 10.1038/s42255-021-00481-w.
PubMed ID: 34697471

Brief summary: This study identified a genetic locus on mouse chromosome 17, containing the gene *Ndufv2*, that controls mitochondrial mass and function in adipose tissue in a sex- and tissue-specific manner. In female mice, *Ndufv2* regulated the expression of 89 mitochondrial genes, with involvements in oxidative phosphorylation and mitochondrial DNA content.

These authors identified a female-specific autosomal locus, which regulates the adipose tissue expression of ∼10% of the 1200 mitochondrial genes. Many of the regulated genes are involved in oxidative phosphorylation or mitochondrial DNA content. This genetic locus showed female-specific associations with obesity-related traits.

Although sex-specific biological mechanisms are to be expected, for too long, animal models and also human clinical trials often focused on only one sex (usually males) to achieve 'greater efficiency' by reducing one possible source of heterogeneity. In March 2022, the UK Medical Research Council (MRC) published a Summary Report on 'Sex in experimental design', which signalled the intent of this national funding body to mandate the inclusion of both sexes by default in all of its funded research, whether in humans, animals or cell-based (1). It warned that research proposals that fail to recognise the importance of sex will likely be rejected. They acknowledged the additional costs of conducting larger experiments and committed to providing such increased funding as long as studies are robustly designed.

Similarly, when we read research papers, we should look out to see whether any findings have been shown to affect both sexes equally or display evidence of being sex-specific.

Reference

1. https://www.ukri.org/publications/sex-in-experimental-design-summary-report/

15.18. Life histories of myeloproliferative neoplasms inferred from phylogenies

Williams N, Lee J, Mitchell E, Moore L, Baxter EJ, Hewinson J, Dawson KJ, Menzies A, Godfrey AL, Green AR, Campbell PJ, Nangalia J
Nature. 2022;602(7895):162-8.
DOI: 10.1038/s41586-021-04312-6.
PubMed ID: 35058638

Brief summary: This study performed whole-genome sequencing (WGS) of 1013 clonal haematopoietic cell colonies from 12 adult patients aged 20–81 years with myeloproliferative neoplasms, a form of blood cancer. They identified 580 133 somatic mutations and used these to reconstruct haematopoietic clonal histories. Key somatic (i.e. non-germline) driver mutations were estimated to have occurred early in life, including fetal life and early childhood.

These remarkable findings extend the list of possible mechanisms linking environmental exposures during early life, including *in utero* development, to risks of diseases they are typically thought of as ageing processes. The mechanism highlighted here is the acquisition by cells of somatic mutations in their DNA sequence, which they show can happen many decades before the manifestation of disease.

One key driver mutation, *JAK2V617F* was estimated to have been acquired from 33 weeks gestation onwards. Another, *DNMT3A* mutations was acquired from 8 weeks of gestation onwards, and a *PPM1D* mutation was acquired by age 5.8 years. The duration between *JAK2V617F* acquisition and disease diagnosis was on average 30 years (range 11–54 years), during which time cells carrying these mutations proliferate extensively giving rise to distinct 'clones of cells', and the subsequent acquisition of additional driver mutations is separated by decades across life.

These findings identify a life-long pathogenesis of adult myeloproliferative neoplasms. Other types of age-related genomic events that lead to clonal expansions, such mosaic loss of chromosome Y, can have impacts on more diverse cancer and non-cancer diseases (1). Identification of the triggers for these somatic mutations and their detection may provide new avenues for early life disease prediction and prevention.

Reference

1. Sano S, *et al*. Hematopoietic loss of Y chromosome leads to cardiac fibrosis and heart failure mortality. *Science* 2022; 377(6603);292–297.

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