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Book Chapters: Darendeliler F. Growth Hormone Treatment in Rare Disorders: The KIGS Experience. In: Ranke MB, Price DA, Reiter EO (eds). Growth Hormone Therapy in Pediatrics: 20 Years of KIGS. Basel, Karger, 2007;213-239.

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Reviews

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Dear Colleagues,

This special issue of The Journal of Clinical Research in Pediatric Endocrinology (JCRPE) entitled "Pediatric Endocrinology update 2017" is intended to provide our readers with information on some of the latest developments and newest clinical practice recommendations in certain areas of pediatric endocrinology. Knowledge in the field of pediatric endocrinology is growing fast, owing mostly to booming data accumulation due to the increasing use of next generation sequencing techniques in molecular biology which have expanded the etiologic spectrum of many endocrinologic diseases, such as hypogonadotropic hypogonadism, adrenal insufficiency and congenital hyperinsulinemia. On the other hand, data coming from contemporary studies have highlighted the need for new nomenclatures in certain areas such as pseudohypoparathyroidism, or a re-evaluation of clinical practice in conditions such as "central precocious puberty" and "metabolic syndrome and insulin resistance". This special issue is intended to supply the reader with a balanced blend of reviews in selected areas of pediatric endocrinology, covering both these rapidly changing areas.

I am indebted to a distinguished list of authors who have devoted their precious time to fulfilling this aim and who have made this special issue possible.

I wish you a happy and prosperous new year.

Abdullah Bereket MD, Guest Editor

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22th National Congress of Pediatric Endocrinology and Diabetes
18-22 April 2018, Belek/Antalya, Turkey

20th International Conference on Pediatrics & Primary Care
3-4 September 2018, Zurich, Switzerland

REVIEW

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Novel Modulators of the Growth Hormone - Insulin-Like Growth Factor Axis: Pregnancy-Associated Plasma Protein-A2 and Stanniocalcin-2

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Abstract

Growth hormone (GH) and its mediator, insulin-like growth factor-1 (IGF-1), play a critical role in human growth. In circulation, IGF-1 is found in a ternary complex with IGF binding proteins (IGFBPs) and acid labile subunit (ALS) but little attention has been paid to the regulation of IGF-1 bioavailability. Recently, pregnancy-associated plasma protein-A2 (PAPP-A2) and stanniocalcin-2 (STC2) were identified as novel modulators of IGF-I bioavailability. PAPP-A2 is a protease which cleaves IGFBP-3 and -5, while STC2 inhibits PAPP-A and PAPP-A2 activity. In collaboration with a group in Madrid, we reported the first human cases carrying mutations in the *PAPPA2* gene who presented with short stature, elevated total IGF-1, IGFBP-3, IGFBP-5 and ALS, but low free IGF-1. Additionally, the patients demonstrated insulin resistance and below average bone mineral density (BMD). The PAPP-A2 deficient patients were treated with recombinant human IGF-1, resulting in improvements in growth velocity, insulin resistance, and BMD. These findings suggested that the bioactive, free IGF-1 liberated from IGFBPs by PAPP-A2 is important for human growth. Mouse models of PAPP-A2 and STC2 provide further insights into their roles in growth physiology. This review will summarize new insights into PAPP-A2 and STC2 and their role in the GH-IGF axis, thereby highlighting the importance of the regulation of IGF-1 bioavailability in human health and disease.

Keywords: Pregnancy-associated plasma protein-A2, stanniocalcin-2, insulin-like growth factor-1, growth hormone

Introduction

Short stature is a very common complaint usually seen by pediatric endocrinologists. The growth hormone (GH) - insulin-like growth factor 1 (IGF-1) axis plays a central role in childhood growth (Figure 1). Human genetic defects affecting this axis lead to a variety of growth disorders (1) and have provided a wealth of knowledge about growth biology. Recently, human genetic studies have pointed to the importance of new components of this axis affecting the regulation of IGF-1 bioavailability. In this article, we will focus on two genes which play critical roles in regulating IGF-1 bioavailability, pregnancy-associated plasma protein-A2 (*PAPP-A2*) and stanniocalcin-2 (*STC2*). We will review the two genes followed by lessons learned from genome-wide association (GWA) studies of adult height in the general population. We will then discuss the recently discovered human mutations in *PAPP-A2* and conclude with a brief review of what has been learned from animal models of these two genes.

Novel Members of the Growth Hormone - Insulin-like Growth Factor System - PAPP-A2 and STC2

The *PAPP-A2* gene (chromosome 1q25.2) encodes the pregnancy-associated plasma protein-A2, a member of the pappalysin family of metzincin metalloproteinases. PAPP-A2 cleaves IGF binding proteins 3 and 5 (IGFBP-3 and IGFBP-5) thereby liberating IGF-1 from its ternary complex which leads to increased, bioactive, free IGF-1 (Figure 2) (2,3). PAPP-A2 protein is widely expressed in human tissues, especially in the placenta and is detected at high levels in the circulation of pregnant women during the first trimester and at term (4). PAPP-A2 is 46% homologous with the closely related protein PAPP-A.

The STC family of proteins has two members, STC1 and STC2, both of which are highly conserved from fish to higher

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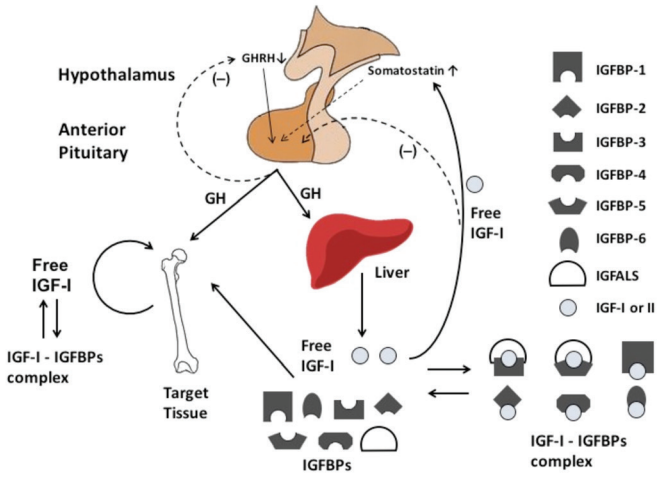

Figure 1. Schematic of the growth hormone - insulin-like growth factor-1 axis in human growth. Growth hormone secretion produces insulin-like growth factor-1 in the liver and at the local target tissue, such as the growth plate. Growth hormone also regulates the expression of insulin-like growth factor binding proteins and insulin-like growth factor acid labile subunit from the liver. Insulin-like growth factor-1 circulates bound to insulin-like growth factor binding proteins and insulin-like growth factor acid labile subunit in serum. Free insulin-like growth factor-1 liberated from insulin-like growth factor binding proteins is the active form of the hormone

IGF-1: insulin-like growth factor-1, IGFBP: insulin-like growth factor binding protein, GH: growth hormone, GHRH: growth hormone-releasing hormone, IGFALS: insulin-like growth factor acid labile subunit

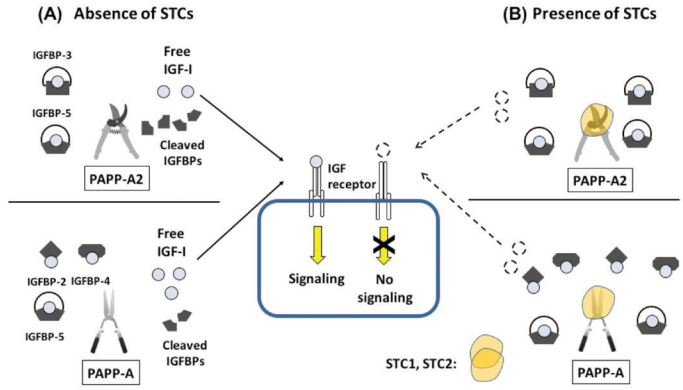

Figure 2. The action of pregnancy-associated plasma protein-A, -A2, and stanniocalcin-1 and -2 on insulin-like growth factor binding proteins in insulin-like growth factor signaling. A) Pregnancy-associated plasma protein-A2 and pregnancy-associated plasma protein-A action without the presence of stanniocalcins. Pregnancy-associated plasma protein-A2 can cleave insulin-like growth factor binding protein-3 and -5 and pregnancy-associated plasma protein-A can cleave insulin-like growth factor binding protein-2, -4, and, -5, resulting in liberation of free insulin-like growth factor-1. Because free insulin-like growth factor-1 can bind its receptor, insulin-like growth factor-1 signaling is then induced. B) Pregnancy-associated plasma protein-A2 and pregnancy-associated plasma protein-A action in the presence of stanniocalcins. Stanniocalcins inhibit pregnancy-associated plasma protein-A2 and -A's ability to cleave insulin-like growth factor binding proteins thereby resulting in decreased levels of free insulin-like growth factor-1 and consequently decreased insulin-like growth factor-1 signaling

IGF: insulin-like growth factor, IGFBP: insulin-like growth factor binding protein, STC: stanniocalcin, PAPP-A: pregnancy-associated plasma protein-A

vertebrates (5). The *STC2* gene is located on chromosome 5 (5q35.2) and is a widely expressed, secreted homodimeric glycoprotein (6). Given STC1's role in calcium and phosphate metabolism, STC2 was first investigated for its putative action on phosphate metabolism and cancer metastasis (7,8,9), but it was later found that STC2's main role is as a component of GH-IGF axis. STC2 was found to be a potent inhibitor of both PAPP-A and PAPP-A2 (10,11) and functions by binding with PAPP-A and PAPP-A2 resulting in their inactivation (Figure 2) (10,11).

Evidence from Genome-wide Association Studies

Over the past decade, there have been numerous GWA studies (GWAS) examining the role of common genetic variants in determining disease risk, as well as variation in anthropometric traits such as height and obesity. In 2010, the Genetic Investigation of Anthropometric Traits Consortium performed a GWAS of adult height in 183.727 individuals (12). They found 180 different genomic loci associated with stature. While the genetic variant in these loci only explained approximately 10% of the phenotypic variation in height, a closer evaluation of biological pathways implicated by these loci provided insights into growth biology. For example, a number of genes known to play a role in growth such as the *GH1* gene as well as genes involved in transforming growth factor-β signaling and the growth plate matrix were identified. Interestingly, additional new genes not previously known to be linked to height were highlighted. For the purposes of this review, it is key to note that both *STC2*, *PAPP-A2*, and its related gene *PAPP-A* were identified as being within genome-wide significant loci. While these three genes had previously been linked to the GH-IGF-1 axis, this was the first time that genetic variation in these genes was linked to human height (12).

In a follow up GWAS, the effects of rare and low frequency coding variants on human height were investigated, as opposed to the previously studied common (allele frequency >5%) non-coding variants. Eighty-three rare and low-frequency coding variants were found to be associated with human height at a genome-wide significant level. Of these 83 variants, the variant with the largest effect size was found in *STC2*. The heights of carriers with this rare *STC2* gene missense variant were approximately 2.1 cm taller than

non-carriers. Functional characterization of the STC2 variant demonstrated that its presence leads to decreased binding of STC2 to PAPP-A *in vitro,* resulting in decreased inhibition of PAPP-A activity and increased cleavage of IGFBP-4 (Figure 3) (13). Presumably, this would result in increased levels of free IGF-1 although this was not directly investigated. This study provides conclusive evidence linking rare damaging variants in STC2 with increased human height.

Rare Mutations in Pregnancy-Associated Plasma Protein-A2 Lead to a Novel Growth Disorder

In 2016, our group, in collaboration with Professor Jesús Argente and his colleagues, reported the first two families with rare damaging mutations in *PAPPA2* (14). We performed whole-exome sequencing in two families of Spanish and Palestinian ancestry whose children presented with short stature and markedly elevated IGF-1 levels. The families were found to be homozygous for the p.D643fs25 and p.A1033V variants in *PAPPA2* respectively (14). Functional studies demonstrated absent expression of the p.D643fs25 mutant at the protein level and significantly reduced expression of the p.A1033V mutant. Importantly, the *PAPPA2* p.A1033V mutant was unable to cleave IGFBP-3 and IGFBP-5 confirming the loss-of-function effect of this mutation.

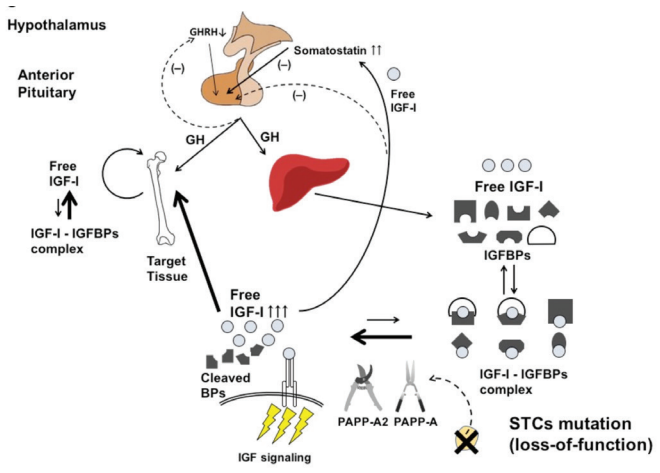

Figure 3. Schematic of the predicted pathophysiology of stanniocalcin-2 deficiency. Mutations which decrease stanniocalcin-2 activity result in decreased inhibition of pregnancy-associated plasma protein-A and pregnancy-associated plasma protein-A2. Therefore, increased protease activities of pregnancy-associated plasma protein-A and -A2 against insulin-like growth factor binding proteins would increase the availability of free bioactive insulin-like growth factor-1. This would be predicted to lead to increased insulin-like growth factor-1 signaling and taller stature

IGF-1: insulin-like growth factor-1, GH: growth hormone, GHRH: growth hormone-releasing hormone, BPs: binding proteins, IGFBPs: insulin-like growth factor binding proteins, PAPP-A: pregnancy-associated plasma protein-A, STCs: stanniocalcins

The Palestinian family had three affected children with significant short stature (height range -2.8 to -3.8 standard deviation scores) while the two affected Spanish children had short stature relative to their mid-parental target height (14). Based on the growth profile of the one post-pubertal patient, it appears that growth failure is progressive and there is no significant pubertal growth spurt. Two of the five affected patients were born mildly small for gestational age. The parents of both families who were heterozygous for the *PAPPA2* mutations were of normal stature. Some of the patients with the homozygous *PAPPA2* mutations also presented with moderate microcephaly, small chin, long thin bones, decreased bone mineral density (BMD) and delayed dental eruption. Biochemically, they had elevated total IGF-1, IGFBP-3, IGFBP-5, acid labile subunit and IGF-2 levels, most of which are GH-dependent factors. The bioactive and free IGF-1 levels were either frankly low or in the low-normal range with a marked decrease in the bioactive/total IGF-1 ratio. GH secretion was elevated in the patients. Presumably, PAPP-A2 dysfunction leads to decreased free IGF-1 levels thus resulting in increased circulating GH concentrations due to a lack of negative feedback on GH secretion (Figure 4).

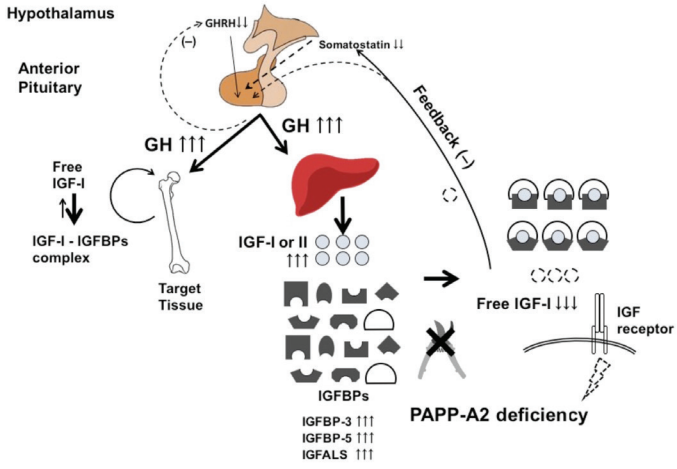

Figure 4. Schematic of the pathophysiology of loss of pregnancy-associated plasma protein-A2 activity. The decreased or mutated pregnancy-associated plasma protein-A2 cannot proteolyze insulin-like growth factor binding protein-3 and -5 resulting in decreased free insulin-like growth factor-1. The reduction of free insulin-like growth factor-1 leads to increased growth hormone secretion due to a lack of negative feedback. Elevated growth hormone levels result in increased production of insulin-like growth factor-1 and -2 and insulin-like growth factor binding proteins. Despite elevation of these hormones, insulin-like growth factor-1 signaling is decreased due to the low levels of free insulin-like growth factor-1

GH: growth hormone, IGF: insulin-like growth factor, IGFBP: insulin-like growth factor binding protein, GHRH: growth hormone-releasing hormone, PAPP-A2: pregnancy-associated plasma protein-A2

Given the decreased levels of free IGF-1 present in these patients, it was hypothesized that treatment with recombinant human IGF-1 (rhIGF-1) could potentially be beneficial for these patients. This approach was first reported by Muñoz-Calvo et al (15) for the two Spanish patients carrying mutant *PAPPA2* p.D643fs25. The rhIGF-1 treatment was administered at a dose of 40-80 μg/kg twice daily for six months. Subsequently, the dose was gradually increased to 120 μg/kg for a total treatment period of one year. The treatment was started at ages 10.5 and six years of age, respectively. Both siblings increased their height by 0.4 standard deviation (SD). Of note, the older girl also received gonadotropin-releasing hormone analog therapy to suppress pubertal development as she entered puberty six months into treatment. Her height velocity increased from 3.7 cm/year (-1.5 SD) pre-treatment to 7.6 cm/year (+1.6 SD) on rhIGF-1 treatment. The younger brother's height velocity also increased from 5.8 cm/year (-1.6 SD) pre-treatment to 7 cm/year (+1.1 SD) on rhIGF-1 treatment (15). For the Palestinian family, the two younger patients carrying the *PAPPA2* p.A1033V mutation were treated with 120 mg/kg of rhIGF-1 (16). The youngest sibling's height increased by 0.4 SD over a period of one year with a doubling of his height velocity from 3 cm/year pre-treatment to 6.2 cm/year on treatment. Unfortunately, the older brother developed severe headaches caused by increased intracranial hypertension, presumably due to the rhIGF-1 treatment, leading to the discontinuation of therapy. After stopping the rhIGF-1 treatment, his symptoms completely resolved (16). His height SD declined from -2.9 to -3.0 over the year despite progressing in pubertal development.

In addition to the effects on height, the subjects have been investigated for the effects of PAPP-A2 deficiency on metabolic parameters and bone health. The three Palestinian children underwent oral glucose tolerance testing and were found to have significant insulin resistance and pre-diabetes. Interestingly, the youngest sibling had complete resolution of the insulin resistance after one year of treatment with rhIGF-1. One possible explanation is that the medication increased free IGF-1 levels thus resulting in lower GH levels with a consequent decrease in insulin resistance. The three affected individuals also had below average BMD with the youngest sibling having an increase in BMD in response to rhIGF-1 therapy.

Characteristics of Pregnancy-associated Plasma Protein-A, Pregnancy-Associated Plasma Protein-A2, Stanniocalcin-1, and Stanniocalcin-2 in Mouse Models

Numerous studies have been performed using knock-out (KO) and transgenic (Tg) mouse models to understand the physiology of PAPP-A, PAPP-A2, STC1, and STC2. Many of the findings seen in these *in vivo* mouse models mimic the features observed in the patients with PAPP-A2 mutations and deepen our understanding of the role that this family of genes play in growth biology. We summarize the phenotypic characteristics of these mouse models in growth, biochemistry, glucose metabolism and bone development in Table 1.

As a first step in understanding the roles of PAPP-A, PAPP-A2, STC1, and STC2 in regulating growth and the GH-IGF axis, anthropometric data of generated mouse models were examined. Homozygous *Pappa*, *Pappa2*, *Stc1*, and *Stc2* KO mice are all viable. In contrast, human *STC2* (hSTC2) Tg mice in which h*STC2* was overexpressed had decreased viability with 26-34% neonatal mortality without apparent dysmorphology (17). Homozygous PAPP-A KO as well as h*STC1* and h*STC2* Tg over-expression mice showed approximately a 30-40% reduction in birth weight relative to wild-type (WT) mice (6,17,18). All three of these models should lead to decreased IGF-1, and possibly IGF-2, bioavailability which is consistent with the decreased birth size. At the other end of the spectrum, homozygous *Stc2* KO mice, which should have increased IGF-1 bioavailability, were born with a birth weight that was 15% heavier than WT (5). Interestingly, there was no significant difference in birth weight between homozygous *Pappa2* or *Stc2* KO mice and WT (19,20). As noted above, only two of the five patients with *PAPPA2* mutations were born small for gestational age, suggesting that perhaps there is a mild effect of PAPPA2 on *in utero* growth in humans that was not present in the current mouse model. Furthermore, the growth limiting (*Pappa* KO, *Pappa2* KO, *STC1* and *STC2* overexpression) and growth promoting (*Stc2* KO) effects of all KO and Tg mice persisted or became more apparent in post-natal growth (Table 1). *Stc1* KO mice remained the same size as WT mice throughout their lives suggesting that STC1 plays a less important role in growth physiology. These results hint at the possibility that these genes could have differential roles in pre- and post-natal growth.

Total IGF-1 values, but not free bioactive IGF-1 were measured in all of the mouse models. Consistent with the human *PAPPA2* mutation patients, total IGF-1 values were higher than WT in homozygous *Pappa2* KO and male h*STC2* Tg mice but not female h*STC2* Tg mice (6,21). In the remaining mouse models, there were no differences in total IGF-1 between mutants and WT (5,17,18). Free IGF-1 values which were measured in the *Pappa2* KO mice were decreased (14). In future studies, it will be important to measure free IGF-1 levels in the other mouse models. There is limited additional data regarding the other biochemical marker of the GH-IGF axis, such as GH and IGFBPs (Table 1). The homozygous *Pappa2* KO animals had increased

Table 1. Phenotypic description of pregnancy-associated plasma protein-A, pregnancy-associated plasma protein-A2, stanniocalcin-1, and stanniocalcin-2 mouse models

	Pappa KO	*Pappa2* KO	*Stc1* KO	*Stc2* KO	h*STC1* Tg (overexpression)	h*STC2* Tg (overexpression)
Birth weight	60-70% of WT	No difference with WT	No difference with WT	115% of WT	70% of WT	70% of WT
Body weight at adult	60% of WT	90% of WT (M), 70-75% of WT (F)	No significant difference with WT	105-119% of WT	50-70% of WT	53-59% of WT
Body length at adult	88% of WT (M)	90% of WT	N/A	Significantly larger than WT	75-83% of WT in young adult	83% of WT
GH	N/A	N/A	No significant difference with WT	N/A	No significant difference with WT	N/A
Total IGF-I in serum	No difference with WT	Significantly higher than WT	No significant difference with WT	No significant difference with WT	No significant difference with WT	Significantly higher in male Tg than WT. Higher in Tg female than WT, but not significantly different when compared with WT
IGFBPs in serum	N/A	Increased IGFBP-5 compared to WT, variable level of BP-3. No difference in BP-2 and -4	N/A	N/A	N/A	N/A
Glucose metabolism	N/A	Not affected	N/A	Not affected	N/A	N/A
Bone	Embryonic delay in bone mineralization, intramembranous and endochondral bone formation	Delayed or no difference in bone development. No difference with WT in BMD	No difference with WT in BMD and bone development		Embryonic delay in bone development in intramembranous and endochondral born formation. The linear axial skeletal growth in long bones was severely compromised in post-natal development	
Reference	Conover et al (18)	Christians et al (20,21,22), Conover et al (23)	Chang et al (5,19)	Chang et al (5)	Gagliardi et al (6), Varghese et al (17), Johnston et al (24)	Gagliardi et al (6), Johnston et al (24)

PAPP-A: pregnancy-associated plasma protein-A, STC: stanniocalcin, KO: knock-out, Tg: transgenic, IGF: insulin-like growth factor, WT: wild type, N/A: not available, GH: growth hormone, BP: binding protein, BMD: bone mineral density, IGFBPs: IGF binding proteins, hSTC1: human STC1, hSTC2: human STC2, M: male, F: female

IGFBP-5 levels and variable levels of IGFBP-3 compared with WT (21,22). There was no difference in IGFBP-2 and -4 when these mice were compared with WT (22).

It is well known that IGF-1 has insulin-like activity acting via the insulin receptor and hybrid insulin/IGF-1 receptors. However, there is little data about glucose metabolism in the KO and Tg mice. In homozygous *Pappa2* KO mice, the blood glucose levels at baseline and during an intraperitoneal glucose tolerance test did not differ from WT mice (21).

Previous studies have shown that IGF-1, as well as IGFBPs,

play a critical role in skeletal growth and maintenance (25,26). Similar to what was seen in the *PAPPA2* mutation patients, *Pappa* and *Pappa2* KO mice showed delayed bone development with regards to bone formation and/or mineralization (Table 1). Interestingly, h*STC1* and h*STC2* Tg mice had severe impairment in post-natal, linear axial skeletal growth (24). No significant effects were seen in the *Stc1* and *Stc2* KO mice.

Conclusion

Since the year 2000, PAPP-A, PAPP-A2, STC1, and STC2 have been highlighted as new players in regulating IGF-1 bioavailability and thus human growth. Our group previously reported the first PAPP-A2 deficiency cases which had short stature together with abnormal glucose and bone metabolism (14). These patients represent a severe perturbation in the regulation of IGF-1 bioavailability and thus provide insights into the importance of this pathway for growth. Additionally, both common and rare genetic variants in this pathway, found in the general population, have been shown to affect adult height. To date, there have been no reports of human patients with severe *STC2* pathogenic mutations. Loss-of-function mutation in *STC2* would be expected to cause tall stature while gain-of-function mutations may cause short stature. The KO and Tg mouse models of these genes, as summarized above, are useful tools to probe the fundamental physiology of these novel growth modulators. However, there are still many unanswered questions for future investigations. Finally, there is minimal data about normal levels of PAPP-A2 or STC2 from fetus to adulthood or how their genes' expression may be regulated. Further studies assessing the roles of PAPP-A2 and STC2 in human growth and bioactive free IGF-1 availability will provide important insights into growth physiology.

Acknowledgements

Andrew Dauber and Vivian Hwa have a patent for the use of recombinant PAPP-A2 as a growth promoting agent.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Masanobu Fujimoto, Andrew Dauber, Design: Masanobu Fujimoto, Andrew Dauber, Data Collection or Processing: Masanobu Fujimoto, Analysis or Interpretation: Masanobu Fujimoto, Andrew Dauber, Vivian Hwa, Literature Search: Masanobu Fujimoto, Andrew Dauber, Writing: Masanobu Fujimoto, Andrew Dauber, Vivian Hwa.

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Latest Insights on the Etiology and Management of Primary Adrenal Insufficiency in Children

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Abstract

Primary adrenal insufficiency (PAI) is a heterogeneous group of disorders characterized by an impaired production of cortisol and other steroid hormones by the adrenal cortex. Most of the causes of PAI in childhood are inherited and monogenic in origin and are associated with significant morbidity and mortality whenever the diagnosis and treatment is delayed. Therefore, early and accurate diagnosis would allow appropriate management for the patients and genetic counselling for the family. Congenital adrenal hyperplasia accounts for most cases of PAI in childhood, followed by abnormalities in the development of the adrenal gland, resistance to adrenocorticotropin hormone action and adrenal destruction. In recent years, the use of genome-wide, next-generation sequencing approaches opened new avenues for identifying novel genetic causes in the PAI spectrum. Understanding the genetic basis of adrenal disorders is key to develop innovative therapies for patients with PAI. The promising progress made in congenital adrenal hyperplasia treatment brings new perspectives for personalized treatment in children with PAI. The aim of this review is to characterize recent advances in the genetics and management of PAI in children.

Keywords: Primary adrenal insufficiency, children, etiology, treatment

Introduction

Primary adrenal insufficiency (PAI) is a relatively rare but potentially lethal clinical condition in which the adrenal cortex cannot produce adequate amounts of steroid hormones, primarily cortisol, but may also include impaired production of aldosterone and adrenal sex steroids. Recent molecular advances have expanded our knowledge of the etiologies of PAI. However, its diagnosis may be missed or delayed unless an illness or stress precipitates a severe cardiovascular collapse resulting in acute adrenal crisis. Early recognition of the clinical findings and treatment with glucocorticoids and rehydration with intravenous fluids, with or without mineralocorticoids and salt, are life-saving while attempts to confirm the diagnosis with extensive work-up are ongoing. Delay in treatment may result in disastrous clinical outcomes.

This review mainly focuses on the recent advances in the etiology, clinical manifestations and management of PAI of genetic origin in children.

Etiology

PAI in children may arise from abnormalities in the development of adrenal gland, impaired steroidogenesis, resistance to adrenocorticotropin hormone (ACTH) action [familial glucocorticoid deficiency (FGD)] or adrenal destruction. In contrast to the predominance of autoimmune etiologies in adults, most causes of PAI in childhood are inherited and monogenic in origin (1,2,3,4). Table 1 summarises the aetiologies of inherited PAI in children.

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH), which occurs in 1 in 10.000-18.000 live births, accounts for most cases of PAI in childhood (5). CAH represents a group of autosomal recessive disorders associated with deficiencies in the enzymes and cofactor proteins required for cortisol biosynthesis (6). Cortisol deficiency increases ACTH production that subsequently leads to adrenocortical hyperplasia and accumulation of the upstream precursor steroids above the enzyme deficiency. The accumulated upstream steroids and their urinary metabolites present the

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Table 1. Aetiologies of inherited primary adrenal insufficiency in children

Condition/deficiency	Gene	OMIM	Associated clinical signs and symptoms
Impaired steroidogenesis			
Impaired cholesterol transport			
Steroidogenic acute regulatory protein (congenital lipoid adrenal hyperplasia)±	*StAR*	201710	46,XY DSD, gonadal insufficiency
Steroidogenic enzyme / co-factor deficiency causing congenital adrenal hyperplasia			
21α-hydroxylase deficiency	*CYP21A2*	201910	46,XX DSD, hyperandrogenism
11β-hydroxylase deficiency	*CYP11B1*	202010	46,XX DSD, hyperandrogenism, arterial hypertension
17α-hydroxylase deficiency	*CYP17A1*	202110	46,XY DSD, arterial hypertension, gonadal insufficiency
P450 oxidoreductase deficiency	*POR*	201750	46,XX and 46,XY DSD, gonadal insufficiency, bone malformations, affects all endoplasmic CYP450 enzyme functions
3β-hydroxysteroid dehydrogenase type 2	*HSD3B2*	201810	46,XX and 46,XY DSD, premature adrenarche, hyperandrogenism in female
P450 side-chain cleavage enzyme (P450scc)	*CYP11A1*	118485	46,XY DSD, gonadal insufficiency
Defects in cholesterol synthesis or metabolism			
Smith-Lemli Opitz disease	*DHCR7*	270400	Mental retardation, craniofacial malformations, growth failure
Abetalipoproteinemia$^{\Omega}$	*MTP*	200100	Ataxia, retinopathy, acanthocytosis, malabsorption of fat
Familial hypercholesterolemia$^{\Omega}$	*LDLR*	143890	Tendon xanthomas, xanthelasma, corneal arcus
Sitosterolemia (phytosterolemia)$^{\Omega}$	*ABCG5* *ABCG8*	210250	Short stature, gonadal failure, xanthomas, hemolytic anemia, arthritis, accelerated atherosclerosis and premature cardiac death
Adrenal dysgenesis/hypoplasia			
Without syndromic features			
X-linked adrenal hypoplasia congenital	*NR0B1 (DAX1)*	300200	Hypogonadotropic hypogonadism in boys. In some cases gonadotropin independent precocious puberty
Xp21contiguous gene deletion syndrome (5 % of cases)	Deletion of genes for Duchenne muscular dystrophy, glycerol kinase, and NR0B1		Duchenne muscular dystrophy, glycerol kinase deficiency, psychomotor retardation (if deletions extend to the *IL1RAPL1* gene)
Adrenal hypoplasia steroidogenic factor-1 deficiency	*NR5A1 (SF1)*	184757	46,XY and 46,XX sex reversal, 46,XY DSD, 46,XX DSD, primary ovarian failure, spermatogenic failure
With syndromic features			
IMAGe syndrome	*CDKN1C*	300290	Intrauterine growth retardation, metaphyseal dysplasia, adrenal insufficiency, genital anomalies
MIRAGE syndrome	*SAMD9*	617053	Myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy
Pallister-Hall syndrome	*GLI3*	165240	Hypothalamic hamartoblastoma, hypopituitarism, imperforate anus, mesoaxial and postaxial polydactyly, laryngotracheal cleft, bifid epiglottis
Meckel syndrome	*MKS1*	249000	Central nervous system malformation (occipital encephalocele), polycystic kidney, hepatic fibrosis, polydactyly
Pena-Shokeir syndrome 1	*DOK7*	208150	Arhrogryposis, fetal akinesia, intrauterine growth retardation, cystic hygroma, pulmonary hypoplasia, cleft palate, cryptorchidism, cardiac defects, camptodactyly, polyhydramnios, intestinal malrotation, pterygiums in extremities

Table 1. Continue

Condition/deficiency	Gene	OMIM	Associated clinical signs and symptoms
Pseudotrisomy 13	*RAPSN*	264480	Holoprosencephaly, facial abnormalities, postaxial polydactyly
Hydrolethalus syndrome	*HYLS1*	236680	Prenatal-onset severe hydrocephalus, polydactyly, micrognathia, abnormal genitalia, congenital heart and pulmonary defects,
Galloway-Mowat syndrome	*WDR73*	251300	Early-onset severe encephalopathy, severe epilepsy, nephrotic syndrome, microcephaly, hiatal hernia
Chromosomal abnormalities	Tetraploidy, triploidy, trisomy 18, trisomy 21, 5p dup, and 11q syndrome		Often associated with central nervous system abnormalities and prenatal-onset growth retardation
ACTH resistance			
Familial glucocorticoid deficiency type 1	*MC2R*	202200	*Generally* isolated glucocorticoid deficiency without mineralocorticoid deficiency, tall stature, subclinical hypothyroidism, characteristic facial features, such as hypertelorism, medial epicanthus and frontal bossing
Familial glucocorticoid deficiency type 2	*MRAP*	607398	*Generally* isolated glucocorticoid deficiency without mineralocorticoid deficiency
Adrenal destruction			
Impaired redox homeostasis			
Nuclear envelope defects			
Triple A syndrome (Allgrove syndrome)	*AAAS*	231550	Alacrimia, achalasia, dysfunction of autonomic nervous system; additional symptoms, including neurologic impairment, deafness, mental retardation, hyperkeratosis
Mitochondrial defects			
Nicotinamide nucleotide transhydrogenase deficiency	*NNT*	614736	*Generally* isolated glucocorticoid deficiency without mineralocorticoid deficiency, subclinical hypothyroidism, insulin-dependent diabetes mellitus
Thioredoxin reductase deficiency[§]	*TXNRD2*	606448	Isolated glucocorticoid deficiency
Glutathione peroxidase deficiency + peroxiredoxine deficiency*	*GPX1 + PRDX3*		A single patient with homozygous gene defects in both genes was described. Patient had isolated glucocorticoid deficiency
Defects in complex lipid metabolism			
a) Peroxisomal defects			
X-linked adrenoleukodystrophy (X-linked ALD)	*ABCD1* *ABCD2*	300100 300371 601081	Progressive neurodegeneration, cognitive and behavioral changes, progressive loss of hearing and vision; dementia, spasticity, seizure
Neonatal adrenoleukodystrophy (autosomal recessive)	*PEX1*	601539	Severe hypotonia, seizures and encephalopathy, blindness and deafness, hepatic dysfunction, peroxysomal agenesis
Zellweger syndrome	*PEX1, 2, 3, 5, 6, 12, 14, 26*	214100	Severe neuromotor and growth retardation, hypotonia, deafness, blindness, craniofacial abnormalities, hepatovmegaly, stippled epiphysis, genitourinary anomalies, infants occasionally mistaken as having Down syndrome
Refsum disease	*PHYH, PEX7*	266500	Multiple epiphyseal dysplasia, cardiomyopathy, anosmia, retinitis pigmentosa, neuropathy, deafness, ataxia, ichthyosis

Table 1. Continue

b) Lysosomal defects			
Wolman disease (lysosomal acid lipase deficiency, cholesterol ester storage disease)	*LIPA*	278000	Diffuse punctate adrenal calcification, xanthomatous changes in liver, adrenals, spleen, lymph nodes, bone marrow, small intestine, lungs and thymus and slight changes in skin, retina, and central nervous system
c) Endoplasmic reticulum defects			
Sphingosine-1-phosphate lyase deficiency	*SGPL1*	603723	Steroid-resistant nephrotic syndrome, ichthyosis, lymphopenia, neurological defects, primary hypothyroidism, cryptorchidism
Autoimmune destruction			
Isolated autoimmune adrenalitis	Association with CLTA-4, HLA-DR3, HLA-DR4, HLA-B8 *BACH2*	-	-
Autoimmune polyglandular syndromes (APS)			
APS type 1	AIRE		Chronic mucocutaneous candidiasis, hypoparathyroidism, other autoimmune disorders, rarely lymphomas
APS type 2	Association with HLA-DR3, CTLA-4		Hypothyroidism, hyperthyroidism, premature ovarian failure, vitiligo, type 1 diabetes mellitus, pernicious anemia
APS type 4	Association with HLA-DR3, CTLA-4 BACH2		Other autoimmune diseases, excluding thyroid disease or diabetes (unusual in children)
Miscellaneous			
DNA repair defects	*MCM4*[¥]	609981	Natural killer cell deficiency, short stature, microcephaly, recurrent viral infections, chromosomal breakage, susceptibility for neoplastic lesions
Bioinactive ACTH*	*POMC*		Signs and symptoms of POMC deficiency (obesity and red hair), high ACTH and low cortisol. Bioinactive but immunoreactive ACTH
Mitochondrial diseases			
Kearns-Sayre syndrome	Mitochondrial DNA deletions, *MTTL1*	530000	Progressive external ophthalmoplegia, ptosis, retinal degeneration, and cardiac conduction defects, microcephaly, other endocrinopathies, lactic acidosis, neuropathy, myopathy, ragged-red fibers seen on muscle biopsy
Mitochondrial DNA polymerase deficiency	*POLG1*	203700	Infantile epilepsy, metabolic strokes, chronic ataxia, neuropathy, and ophthalmoplegia, type I diabetes, hypothyroidism and psychiatric problems
Impaired mitochondrial disulfide relay system	*GFER*	613076	Encephalomyopathy, congenital cataracts, hypotonia, developmental delay and sensorineural hearing loss, lactic acidosis, respiratory failure
MELAS syndrome	*MTTL1, MTTQ, MTTH, MTTK, MTTC, MTTS1-2, MTND1, 5, 6*	540000	Mitochondrial myopathy, encephalopathy, lactic acidosis, *stroke-like episodes*
Impaired complex I assembly	*NDUFAF5*	252010	Agenesis of the corpus callosum and ventricular septation, congenital left diaphragmatic hernia and lactic acidosis

Table 1. Continue

Impaired translation	*MRPS7, QRSL1*	611974, 617209	Sensorineural deafness, primary ovarian failure, progressive hepatic and renal failure and lactic acidemia
Pearson syndrome	Contiguous gene deletion/ duplication of several mtDNA genes	557000	Low birth weight, failure to thrive, sideroblastic anemia, exocrine pancreatic dysfunction

[±]Mild defects may present like familial glucocorticoid deficiency (known mutations of StAR causing non-classic deficiency are p.R192C, p.R188C), [Ω]Asssociated with mild biochemical cortisol deficiency but not clinically significant adrenal failure, [§]Described only in seven individuals from a consanguineous Kashmiri kindred, to date, [¥]Described only in the Irish traveler population, to date, *Described in a single patient

StAR: steroidogenic acute regulatory protein, *CYP21A2*: cytochrome P450, family 21, subfamily A, polypeptide 2, CYP11B1: cytochrome P450, family 11, subfamily B, polypeptide 1, CYP17A1: cytochrome P450, family 17, subfamily A, polypeptide 1, POR: cytochrome P450, oxidoreductase, HSD3B2: 3β-hydroxysteroid dehydrogenase 2, CYP11A1: cytochrome P450, family 11, subfamily A, polypeptide 1, DHCR7: 7-dehydrocholesterol reductase, MTP: microsomal triglyceride transfer protein, LDLR: low density lipoprotein receptor, ABCG8: ATP-binding cassette, subfamily G, member 8, NR0B1: nuclear receptor subfamily 0, group B, member 1, DAX1: dosage sensitive sex reversal, adrenal hypoplasia congenita, critical region on X chromosome, gene 1, NR5A1: nuclear receptor subfamily 5 group A member 1, SF1: steroidogenic factor 1, CDKN1C: cyclin dependent kinase inhibitor 1C, SAMD9: sterile alpha motif domain-containing protein 9, GLI3: gene responsible for Greig cephalopolysyndactyly syndrome, MKS1: gene responsible for Meckel syndrome, type 1 and Bardet-Biedl syndrome type 13, DOX7: docking protein 7, RAPSN: receptor associated protein of the synapse, HYLS1: hydrolethalus syndrome protein 1, WDR73: WD repeat domain 73, MC2R: melanocortin 2 receptor, MRAP: melanocortin 2 receptor accessory protein, AAAS: achalasia-alacrima-addisonianism, NNT: nicotinamide nucleotide transhydrogenase, TXNRD2: thioredoxin reductase 2, GPX1: glutathione peroxidase 3, PRDX3: peroxiredoxin 3, ABCD1: ATP-binding cassette, subfamily D, member 1, PEX: peroxisome biogenesis factor, PHYH: phytanoyl-CoA hydroxylase, LIPA: lipase A, SGPL1: sphingosine-1-phosphate lyase, BACH2: BTB and CNC homology 2, AIRE: autoimmune regulator, MCM4: minichromosome maintenance complex component 4, POMC: proopiomelanocortin, POLG1: polymerase, DNA, gamma-1: GFER1: growth factor, ERV1-like, NDUFAF5: NADH dehydrogenase (ubiquinone) complex I, assembly factor 5, MRPS7: mitochondrial ribosomal protein S7, QRSL1: glutaminyl t-RNA-synthase like protein 1, MIRAGE: myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital

biochemical fingerprints for the localization of the defect (Figure 1). Additionally, these steroid precursors are generally diverted to androgen producing alternate pathways leading to androgen excess. The accumulation of certain steroid precursors enable differentiation of steroidogenic enzyme deficiencies (except StAR and P450 side-chain cleavage enzyme deficiencies) from the rest of the etiologies leading to PAI, as non-CAH is characterized by elevated ACTH concentrations and low steroidogenic intermediates.

The presence of hyperpigmentation of skin, nail beds, mucous membranes, palmar creases and scars is one of the hallmarks of primary adrenocortical pathology. ACTH and alpha-melanocyte stimulating hormone (α-MSH) are cleavage products of pro-opiomelanocortin (POMC). In patients with low cortisol levels as a consequence of adrenal disorders, POMC synthesis and consequently ACTH and MSH levels rise by negative feedback mechanisms. α-MSH then binds to the melanocortin 1 receptor on melanocyte cells, inducing a switch from the production of the pale skin pigment pheomelanin to eumelanin which is the darker (brown or black) pigment (7).

Clinical presentation may be mild or severe depending on the degree of impairment of enzyme activity and there may be signs, symptoms and laboratory findings of cortisol deficiency, mineralocorticoid deficiency or excess, undervirilization or androgen excess in males and sexual infantilism or virilization in affected females. The main signs and symptoms of cortisol deficiency include anorexia, weight loss, fatigue, myalgia, joint pain, low blood pressure, orthostatic hypotension, hyponatremia, hypoglycemia, lymphocytosis and eosinophilia and in addition direct hyperbilirubinemia and apnea may be present in newborn babies. Mineralocorticoid synthesis and release is under the control of the renin-angiotensin system, rather than ACTH. Therefore, mineralocorticoid deficiency develops only in adrenocortical abnormalities. Mineralocorticoid deficiency causes failure to thrive, abdominal pain, nausea, vomiting, dizziness, low blood pressure, orthostatic hypotension, hyponatremia, salt craving, hyperkalemia, metabolic acidosis, dehydration and hypovolemic shock. Lack of pubic and/or axillary hair and absent/delayed clinical adrenarche in either sex suggests deficiency of adrenal sex steroids.

More than 95% of all cases of CAH are caused by 21-hydroxylase deficiency (21-OHD). 21-OHD is classified into 3 subtypes according to retained enzyme activity and clinical severity: classic salt wasting, classic simple virilizing, and nonclassic CAH (NCCAH; mild or late onset) (6,8). The classic type affects approximately 1 in 16.000 live births. NCCAH is one of the most common autosomal recessive disorders in humans and affects approximately 1 in 1000 individuals (6). The second most common form of CAH, 11β-hydroxylase deficiency (11-OHD), occurs in 1 in 100,000 live births and accounts for approximately 5% of cases (9). Other less common forms of CAH include 3β-hydroxysteroid dehydrogenase type 2 deficiency, 17α-hydroxylase deficiency, POR deficiency, lipoid CAH and cholesterol side-chain cleavage enzyme deficiency. Distinctive clinical and biochemical features and management goals of CAH are presented in Table 2. An expert review on the genetic features of CAH is also available (6).

Advances in steroid assays in recent years, particularly the clinical utility of liquid chromatography/tandem mass spectrometry (LC-MS/MS), have allowed more accurate quantitation of key steroids, simultaneous measurement

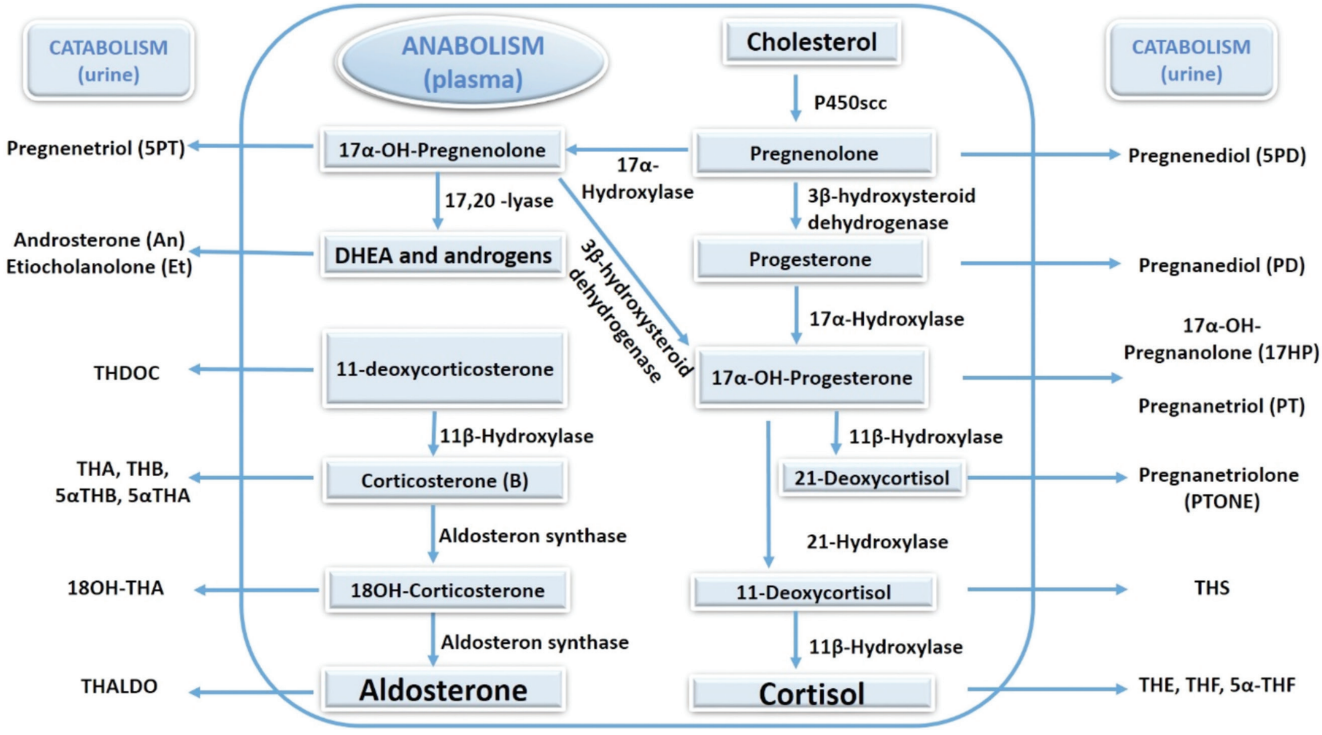

Figure 1. Adrenal steroidogenesis showing the main enzymatic steps of the pathway, intermediate precursors measurable in plasma and their respective catabolic metabolites detectable in the urine

P450scc: P450 side chain cleavage, DHEA: dehydroepiandrosterone, THDOC: tetrahydrodeoxycorticosterone, 18OH-THA: 18OH-tetrahydrodehydrocorticosterone, (5α)THA: (5α)tetrahydrodehydrocorticosterone, (5β)THB: (5β)tetrahydrocorticosterone, THALDO: tetrahydroaldosterone, THS: tetrahydrodeoxycortisol, THE: tetrahydrocortisone, (5α)THF: (5α)tetrahydrocortisol

of multiple steroids from small biological samples and identification of novel steroids in the pathogenesis of adrenal disorders. The best example of this is the emerging evidence of 11-oxygenated 19-carbon (11oxC19) adrenal-derived steroids as clinically important androgens. 11oxC19 steroids are synthesized by the action of cytochrome P450 11β-hydroxylase. Besides the last step of cortisol biosynthesis, cytochrome P450 11β-hydroxylase mediates the conversion of androstenedione and testosterone into their respective 11-oxygenated products, namely 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT). These steroids are further converted to small amounts of 11-ketoandrostenedione (11KA4) and 11-ketotestosterone (11KT) respectively, by the action of 11β-hydroxysteroid dehydrogenase, type 2. 11oxC19 steroids are produced almost exclusively from the adrenal gland and they were shown to be three to four times higher in 21OHD patients than in controls. In addition 11KT was found to be more closely associated with poor control in 21OHD than testosterone levels in both males and females. Therefore it has been hypothesized that 11KT is a major adrenal androgen, responsible for suppression of gonadal functions observed in poorly controlled 21OHD (10). Furthermore, 21-deoxycortisol and 11oxC19 steroids showed the closest correlation with adrenal gland size and 11oxC19 steroids were detected at much higher concentrations in CAH patients with testicular adrenal rest tumor (TART) than those without (11). These findings suggest that 11oxC19 steroids may present clinically promising biomarkers in the treatment monitoring and management of CAH.

Adrenal Dysgenesis/Hypoplasia

During the last two decades, high-throughput sequencing approaches proved very effective in reaching a molecular diagnosis for several forms of primary adrenal hypoplasia and adrenal dysgenesis syndromes. The genetic basis for these disorders involves various cellular and physiologic processes, including metabolism, nuclear protein import, oxidative stress defense mechanisms and regulation of cell cycle (12). Two distinct histological patterns of adrenal hypoplasia have been described; the miniature adult and cytomegalic forms. In the miniature adult form, adrenal cortex has normal structural organization whereas in the cytomegalic form of primary adrenal hypoplasia the residual adrenal cortex is structurally disorganized with scattered irregular nodular formations of eosinophilic cells, with the adult permanent zone absent or nearly absent. The

Table 2. Clinical and laboratory findings of different forms of congenital adrenal hyperplasia and treatment goals

Condition	Clinical landmarks	Impaired steroidogenesis	Steroid status	Laboratory findings	Treatment
21α-hydroxylase deficiency	Cortisol deficiency, mineralocorticoid deficiency (salt-wasting crisis), 46,XX DSD, postnatal virilization in both sexes	Adrenal	Glucocorticoids and mineraocorticoids↓, adrenal sex steroids↑	Serum/plasma: Cortisol↓ ACTH↑ serum basal and ACTH-stimulated 17OHP↑ 21-deoxycortisol*↑, 4AS↑ testosterone↑ hyponatremia, hyperkalemia, plasma renin activity↑ Urine: Pregnanetriolone§↑, Pregnanetriol↑, 17αOH-Pregnanolone↑	Glucocorticoid (hydrocortisone), mineralocorticoid and salt replacement, vaginoplasty, cliteroplasty, suppression of hyperandrogenism by glucocorticoids
11β-hydroxylase deficiency	Cortisol deficiency, 46,XX DSD, postnatal virilization in both sexes, hypertension	Adrenal	Glucocorticoids↓, mineralocorticoids and adrenal sex steroids↑	Cortisol↓ ACTH↑ serum basal and ACTH-stimulated 11-deoxycortisol* and deoxycorticoserone↑, 4AS↑, testosterone↑ hypokalemia, plasma renin activity↓ Urine: Tetrahydrodeoxycortisol§↑	Glucocorticoid (hydrocortisone) replacement, vaginoplasty, cliteroplasty, suppression of hyperandrogenism by glucocorticoids
3β-hydroxysteroid dehydrogenase type 2	Cortisol deficiency, Mineralocorticoid deficiency (salt-wasting crisis) 46,XX and 46,XY DSD, pubertal disorders and premature adrenarche in both sexes	Adrenal, gonadal	Glucocorticoids, mineralocorticoids and adrenal sex steroids↓	Cortisol↓ ACTH↑ serum basal and ACTH-stimulated Δ5 steroids (pregnenolone, 17-hydroxypregnenolone*, DHEA)↑, 4AS↓ testosterone↓ hyponatremia, hyperkalemia, plasma renin activity↑ Urine: Pregnenetriol§↑, pregnenediol↑	Glucocorticoid (hydrocortisone), mineralocorticoid and salt replacement, genitoplasty, sex steroid replacement at puberty, suppression of hyperandrogenism by glucocorticoids
17α-hydroxylase / 17,20 lyase deficiency	Cortisol deficiency (excessive deoxycorticosterone masks clinical findings of glucocorticoid deficiency), 46,XY DSD, Absence of pubertal development, hypertension	Adrenal, gonadal	Glucocorticoids and adrenal sex steroids↓, mineralocorticoids ↑	Cortisol↓ ACTH↑ serum basal and ACTH-stimulated corticosterone and 11-deoxycorticosterone*↑, 17α-hydroxylated steroids↓, 4AS↓ testosterone↓ hypokalemia, plasma renin activity↓ Urine: (5α)Tetrahydrodehydrocorticosterone↑, (5β)Tetrahydrocorticosterone↑ Androsterone↓, Etiocholanolone↓	Glucocorticoid (hydrocortisone), replacement, genitoplasty, sex steroid replacement at puberty
Congenital lipoid adrenal hyperplasia (StAR deficiency), P450 side chain cleavage (CYP11A1) deficiency	Cortisol and mineralocorticoid deficiency (salt-wasting crisis), 46,XY DSD, absence of pubertal development and premature ovarian failure in females	Adrenal, gonadal	Glucocorticoids, mineralocorticoids and adrenal sex steroids↓	Cortisol↓ ACTH↑ serum basal and ACTH-stimulated steroids and their precursors are low, hyponatremia, hyperkalemia, plasma renin activity↑, FSH and LH↑, testosterone and estradiol↓	Glucocorticoid (hydrocortisone), mineralocorticoid and salt replacement, genitoplasty, sex steroid replacement at puberty
P450 oxidoreductase deficiency	Cortisol deficiency, 46,XX and 46,XY DSD, Antley-Bixler syndrome, maternal virilization	Adrenal, gonadal		Cortisol↓ ACTH↑ pregnenolone↑, progesterone↑, prenatal androgens↑, androgen and estrogens at puberty↓ Urine: Pregnanetriol↑, 17αOH-pregnanolone↑, androsterone↓, etiocholanolone↓	Glucocorticoid (hydrocortisone) mineralocorticoid and salt replacement, genitoplasty, sex steroid replacement at puberty

*Best diagnostic biochemical marker in serum, §Best diagnostic biochemical marker in urine
ACTH: adrenocorticotropin hormone, StAR: steroidogenic acute regulatory protein, LH: Luteinizing hormone, FSH: follicle stimulating hormone, DHEA: dehydroepiandrosterone

miniature adult form is generally sporadic or inherited in an autosomal recessive manner while the cytomegalic form is generally considered to be X-linked, but there may be one or more autosomal genes associated with this phenotype (12). Regardless of underlying genetic etiology, conditions with adrenal hypoplasia/dysplasia are associated with deficiency of all adrenocortical hormones (aldosterone, cortisol, androgens). Most common is DAX1 deficiency which is due to genetic defects in *NR0B1*, located on chromosome Xp21.2. DAX1 defects have been detected in two thirds of males with PAI of unknown etiology by clinical or biochemical phenotype (13). Therefore all male patients with a history of non-CAH PAI should be screened for DAX1 deficiency, especially those with infertility, delayed/absent puberty or adrenal insufficiency in males from the maternal family. Adrenal insufficiency shows a bimodal distribution pattern of age at presentation ie either around newborn period or after 1 year of age. However late-onset DAX1 deficiency cases are also being increasingly reported from adult clinics (3,14). Patients with DAX1 deficiency present with variable phenotypes. Typically, they develop severe primary adrenal failure with salt-wasting. The hypogonadotropic hypogonadism may manifest as delayed puberty, impaired spermatogenesis or infertility which is explained by the expression of *NR0B1* in the hypothalamus and the anterior pituitary, besides the adrenal glands and the gonads. Therefore, long-term focus on puberty and fertility is needed in affected individuals. Ambiguous genitalia is not a feature of DAX1 deficiency. However micropenis and or cryptorchidism may be present. Patients with precocious puberty have also been reported (15,16). Although this is an X-linked condition, females carrying homozygous or heterozygous mutations have also been reported to express phenotypic features of adrenal hypoplasia congenital due to non-random X inactivation (17,18). Genetic counselling can help to identify family members at risk of adrenal insufficiency and female carriers.

The SF1 protein, encoded by the nuclear receptor subfamily 5, group A, member 1 (*NR5A1*) gene, is expressed in the adrenal gland, gonads, hypothalamus and anterior pituitary. SF1 has a crucial role in adrenal gland, gonads and spleen development in both sexes. Besides, SF1 is involved in the regulation of energy balance and glucose homeostasis in the central nervous system (19). SF1 deficiency develops as a result of pathogenic mutations in *NR5A1* gene in both heterozygous or homozygous inheritance. In contrast to DAX1-associated diseases, SF-1 deficiency only rarely causes adrenal insufficiency, but generally in combination with testicular dysgenesis. Isolated adrenal failure has rarely been reported (20). However, long-term follow-up for adrenal function is important for those patients with *NR5A1* mutations. Phenotypic features in 46,XY individuals with *NR5A1* mutations include different forms of disorders of sex differentiation (DSD) ranging from hypospadias to complete female phenotype or late-onset impaired spermatogenesis and infertility. *NR5A1* gene defects should also be considered in 46,XY DSD cases with normal testosterone concentrations, similar to androgen receptor (*AR*) mutations or mild 5-α reductase, or mild 17-ketosteroid reductase deficiencies. Mutations in *NR5A1* were found in 46,XX females with isolated/premature ovarian insufficiency (14). 46,XX testicular/ovotesticular DSD is also described in one case (21,22). Poly/asplenia can be seen in both sexes (23).

The common feature of syndromes associated with adrenal hypoplasia is the severe impairment of growth and tissue development and particularly with a prenatal onset. These disorders specifically impair the machinery involved in cell division and cell cycling. The author suggests evaluation of adrenal function in any patient with severe, prenatal-onset growth retardation and with syndromic features, especially with cerebral and finger malformations (Table 1).

Here, two specific examples of syndromic adrenal hypoplasia are given.

IMAGe syndrome is a recently described, syndromic adrenal hypoplasia syndrome associated with severe growth failure. This syndrome develops as a result of impaired expression of a cell cycle regulator protein, cyclin dependent kinase inhibitor 1C (CDKN1C). CDKN1C, encoded by the *CDKN1C* gene, is a negative regulator of cell proliferation maintaining the cell at the non-proliferative state throughout life. The loss-of-function mutations, located at the CDK-binding domain of the *CDKN1C* gene, are associated with Beckwith-Wiedemann syndrome. Recently, gain-of-function mutations in the PCNA domain of *CDKN1C* have been have been described in association with various growth-retarded syndromes including IMAGe syndrome and Russell Silver syndrome as well as a novel undergrowth syndrome that additionally exhibits early adulthood onset diabetes (24). *De novo* heterozygous *CDKN1C* mutations or imprinted mode of inheritance with maternal transmission of *CDKN1C* mutations were reported. Early recognition of metaphyseal dysplasia accompanying early-onset, severe adrenal insufficiency is crucial for the diagnosis IMAGe syndrome. Delayed endochondral ossification, osteopenia, hypercalcemia, and/or hypercalciuria of variable degree are among the early findings. Dysmorphic craniofacial features including prominent forehead, low-set ears, short nose, flat nasal bridge, rhizomelic shortening and genital abnormalities in males are other associated features.

Another severe growth-restricting pathology associated with adrenal hypoplasia has recently been described in patients due to gain-of-function mutations in the *SAMD9* gene. Growth and survival is so impaired in this genetic disorder that affected individuals develop tissue adaptation by progressive loss of mutated *SAMD9* in chromosomal structure. This modification is achieved through the development of monosomy 7 (-7), deletions of 7q (7q-), and secondary somatic loss-of-function (nonsense and frameshift) mutations in *SAMD9* to rescue the growth-restricting effects of mutant SAMD9 proteins in bone marrow and to increase the length of survival (25). So the use of advanced diagnostic and molecular technologies has helped to define novel mechanisms in human development beyond genetic defects in adrenal development and adrenal steroidogenesis.

Affected individuals with heterozygous gain-of-function mutation in *SAMD9* present with MIRAGE syndrome, which is an acronym of myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy (26).

Adrenocorticotropin Hormone Resistance

Mutations in *MC2R* (encoding the ACTH receptor protein, MC2R) and *MRAP* (encoding MC2R accessory protein) are well described causes of inherited disorders of ACTH binding and signaling, namely FGD type 1 (FGD1) and type 2 (FGD2). FGD is characterized by cortisol deficiency together with a preserved renin-aldosterone axis. Children typically present with hypoglycemia or hyperpigmentation in early infancy or in childhood. Some associated phenotypical features may also be present (Table 1). Children with FGD do not typically have salt-loss. However, transient hyponatremia has been reported in several children with severe *MC2R* defects, sometimes leading to a misdiagnosis of adrenal hypoplasia (3). Plasma ACTH often remains markedly raised despite normal or even supranormal glucocorticoid treatment. Therefore, affected patients remain hyperpigmented. So the clinical aim of glucocorticoid replacement strategies should not be to suppress ACTH or normalization of hyperpigmentation but should rather target normal water and electrolyte balance and a normal physical growth rate.

Mitochondria and Adrenal Gland

Recent advances in molecular studies and application of genome-wide, next-generation sequencing approaches revealed the importance of mitochondrial function for endocrine health and steroid hormone biosynthesis. All steroid hormones are synthesized within mitochondria by tissue-specific steroidogenic enzymes (Figure 2). Mitochondrial dysfunction may affect the capacity for adrenocortical hormone production by impaired mitochondrial ATP production, oxidative stress and/or accelerated apoptosis (27). In particular some of the latest findings have expanded the spectrum of pathogenetic mechanisms causing adrenal disease and imply that the adrenal is highly vulnerable to oxidative stresses (Figure 2) (28,29).

Molecular defects in both mitochondrial and nuclear genomes have been associated with mitochondrial dysfunction (Table 1). Clinicians should have a high level of suspicion for the possibility of an underlying mitochondrial disease in patients with adrenal insufficiency associated with sensorineural hearing loss, lactic acidosis and accompanying endocrine abnormalities (diabetes, hypoparathyroidism, hypogonadism, hypothyroidism) and multisystemic diseases (epilepsy, stroke, encephalopathy, cranial abnormalities, cardiac conduction defects, neuropathy, retinopathy).

Sphingolipids and Adrenal Gland

The essential role of sphingolipid metabolism has recently emerged in adrenal disease. Congenital sphingosine1phosphate (S1P) lyase deficiency due to biallelic mutations in the *SGPL1* gene has been described, in association with PAI and steroid-resistant nephrotic syndrome (30,31,32). S1P lyase is the enzyme responsible for irreversible S1P degradation which is the final step in sphingolipid breakdown. Inhibition of S1P lyase activity will lead to accumulation of bioactive signaling molecules upstream of the pathway including S1P and ceramides (Cer). We have recently demonstrated that accumulation of S1P, Cer and potentially other upstream components of the sphingolipid pathway, due to congenital S1P lyase deficiency, leads to a multisystemic disorder including PAI, nephrotic syndrome and ichthyosis, primary hypothyroidism, cryptorchidism, lymphopenia and neurological anomalies.

Establishing a specific genetic diagnosis of PAI is extremely valuable for identifying presymptomatic children who could benefit from treatment before the onset of potentially life-threatening symptoms and for counseling family members appropriately about the risk of passing the condition on to their children. Knowing the genetic etiology can also help to modify treatments, such as the need for long-term mineralocorticoid replacement, and can predict potential co-morbidities, such as impaired puberty or fertility and neurological dysfunction. An etiological approach in children with Inherited Primary Adrenal Insufficiency is suggested in Figure 3.

Treatment

Replacement of glucocorticoids and mineralocorticoids, particularly by hydrocortisone and fludrocortisone is

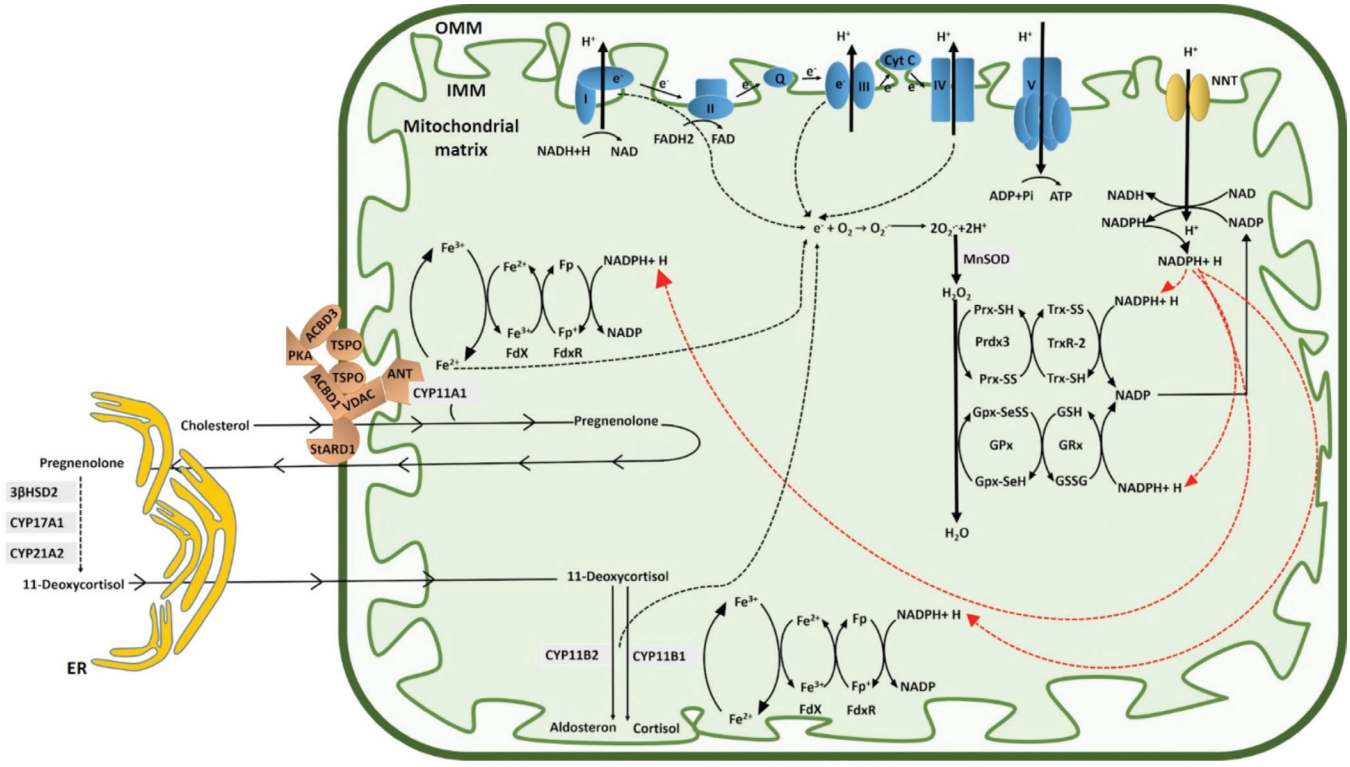

Figure 2. Mitochondrial machinery involved in the regulation of steroidogenesis. Access of cholesterol to the mitochondria is regulated by the steroidogenic acute regulatory protein, (StAR), serving as the acute regulator of steroidogenesis. StAR action requires interaction with the transduceosome complex that is composed of a group of proteins (translocator protein, voltage dependent anion channel 1, ACBD3, adenine nucleotide transporter, protein kinase A) at the inner mitochondrial membrane in the process of transporting cholesterol molecules directly to the cholesterol side chain cleavage enzyme, P450scc (CYP11A1) to initiate steroidogenesis. CYP11A1 is the enzymatic rate-limiting step in steroidogenesis which determines cellular steroidogenic capacity. CYP11A1, CYP11B1 and CYP11B2 are the main mitochondrial cytochrome P450 enzymes involved in steroidogenesis. Four complexes of the electron transport chain (indicated in blue) transfer electrons to generate energy required for various cellular processes including steroid biosynthesis. Nicotinamide nucleotide transhydrogenase (NNT), is an integral protein of the inner mitochondrial membrane. This enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is the electron supplier for two electron-transfer intermediates, ferredoxin reductase and ferredoxin which are required for mitochondrial P450 enzymes to produce steroid hormones. NADPH is also used by mitochondrial antioxidant defence machinery, comprising glutathione peroxidase (Gpx) and the peroxiredoxin-thioredoxin systems, which are responsible for the inactivation of reactive oxygen species derived from the leakage of electrons from electron transport chain during energy generation procedures. Genetic defects in many components of this machinery (including StAR, CYP11A1, CYP11B1, NNT, TXNRD2, GPX1, PRDX3) have been described in patients with primary adrenal insufficiency

TSPO: translocator protein, VDAC: voltage dependent anion channel, ANT: adenine nucleotide transporter, PKA: protein kinase A; FdXR: ferredoxin reductase, FdX: ferredoxin, Gpx: glutathione peroxidase, NADPH: nicotinamide adenine dinucleotide phosphate, NNT: Nicotinamide nucleotide transhydrogenase, StAR: steroidogenic acute regulatory protein, Prdx-Trx: peroxiredoxin-thioredoxin

the mainstay of treatment in adrenal insufficiency. Intravenous fluids and salt replacement should be added to the treatment in stressful conditions and adrenal crisis. Principal treatment goals include maintaining a physiologic water and electrolyte homeostasis together with attainment of normal physical and pubertal growth. CAH management should also target reduction of androgen exposure. Additionally, optimization of hydrocortisone treatment is critical to mimic the physiological circadian rhythm of cortisol secretion and to avoid excessive glucocorticoid exposure which is associated with poor long-term health outcomes, including growth suppression, obesity, metabolic syndrome, diabetes and osteoporosis (33). These challenges have led to the development of new glucocorticoid formulations and some adjuvant treatments (34). In recent years, investigators have developed two modified-release, oral, glucocorticoid preparations. The first is a dual-release hydrocortisone with an extended-release core surrounded by an immediate-release coating (Plenadren; ViroPharma, Maidenhead, UK), which was developed for once-daily, first-morning administration in patients with PAI. However, it is unable to deliver a sufficient early morning cortisol rise and

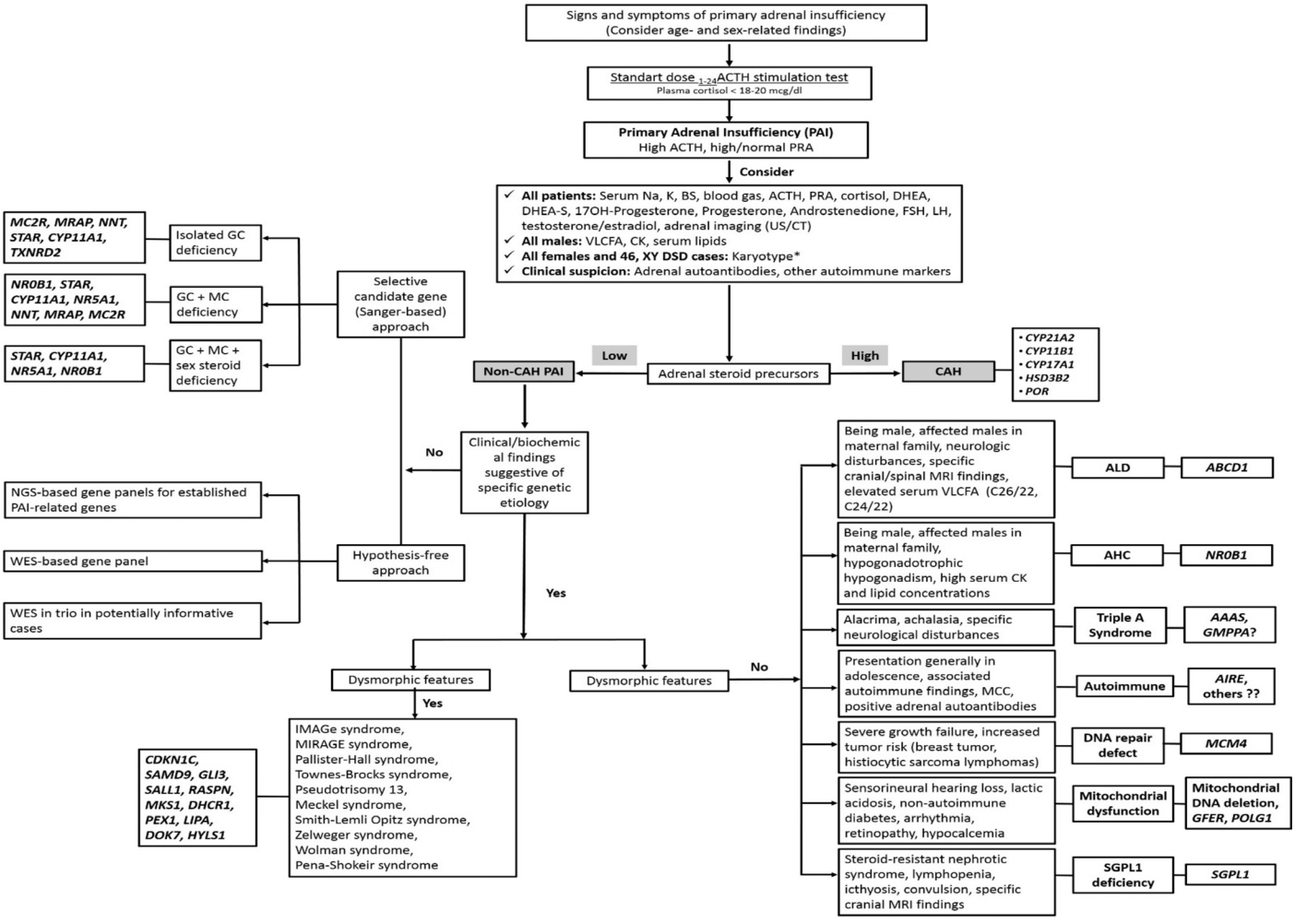

Figure 3. A proposed diagnostic work up algorithm for targetted genetic testing to determine the etiologic diagnosis in inherited primary adrenal failure in children

*Karyotype can be excluded in female phenotype patients whenever pelvic US confirms the presence of normal ovaries and Mullerian structures. Assessment of karyotype-matched normal external and internal genitalia and gonads is crucial for deciding about gonadal sex steroid production

ACTH: adrenocorticotropin, PRA: plasma renin activity, Na: sodium, K: potassium, BS: blood sugar, DHEA: dehydroepiandrosterone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, VLCFA: very-long-chain fatty acids, CK: creatinine kinase, US: ultrasound, CT: computerized tomography, CAH: congenital adrenal hyperplasia, GC: glucocorticoid, MC: mineralocorticoid, MRI: magnetic resonance imaging, ALD: adrenoleukodystrophy, AHC: adrenal hypoplasia congenital, MCC: mucocutaneous candidiasis, NGS: next generation sequencing, WES: whole exome sequencing, SGPL1: sphingosine-1-phosphate lyase

to suppress ACTH and adrenal androgens in the morning by once-daily dosing. Plenadren failed to achieve physiologic cortisol replacement in a small case series of children with non-CAH primary adrenal failure and secondary adrenal insufficiency (35,36,37). Plenadren is not yet licensed for use in the management of adrenal insufficiency in children, but is available for use in adult patients with a good safety profile (38). The second formulation is a delayed and sustained release, multiparticulate hydrocortisone, Chronocort® (Diurnal, UK). Chronocort given at morning and night doses provides release of hydrocortisone in the early hours of the morning, replicating a physiological cortisol secretion pattern. It also appears to achieve better control of excessive androgen synthesis produced via classical and alternative pathways through attenuation of androstenedione and 17OH-progesteron (39). There is an ongoing phase III study to evaluate long-term effects of Chronocort treatment. This drug is also not licenced for use in children. There are a few recent trials to evaluate the bioavailability and absorption of modified hydrocortisone formulations, such as granules or sprinkles, for young children (Infacort®, Diurnal Ltd) (40). Continuous subcutaneous hydrocortisone infusion (CSHI) via a pump, similar to an insulin pump, is superior in achieving a better cortisol secretion profile and lowering ACTH concentrations in non-CAH PAI and in lowering serum androgens in CAH (41,42). However, certain issues limit the

use of CSHI including high cost, complexity of device usage, the need for patient/parent education, the potential for local irritation and the potential for uninterrupted equipment wear and malfunction which would be particularly risky in patients with complete glucocorticoid deficiency. A recent meta-analysis demonstrated that extended-/dual-release and CSHI forms of glucocorticoid treatments are associated with higher life quality scores over the short-term (43).

Non-glucocorticoid adjuvant pharmacologic treatments for adrenal failure mainly target control of hyperandrogenism in CAH (34). Among them, abiraterone may be a promising alternative therapy that decreases the need for supraphysiologic exogenous glucocorticoids. Abiraretone is a potent inhibitor of CYP17A1, required for the synthesis of gonadal and adrenal androgens. Combined use of abiraterone with glucocorticoids can effectively lower androstenedione and testosterone metabolites in adult women with 21OHD without any potential side effects including hypertension and hypokalemia. However, it does not lower ACTH and inhibits gonadal sex-steroid secretion which limits its use in males with TART and for patients who desire fertility (44). A CRH receptor-1 antagonist was used in a Phase 1 trial of eight CAH women at a single dose which showed a 40% reduction in morning ACTH rise to control hyperandrogenism (45).

Conclusion

PAI is a relatively rare but potentially lethal clinical condition in children. Early recognition of adrenal insufficiency can be difficult, although treatment is usually successful once it is initiated and, in most cases, lifelong treatment is necessary. Monogenic conditions, particularly CAH, account for most cases of PAI in childhood. Application of omics-based approaches by LC combined with MS significantly facilitated the recognition of biochemical markers of various steroidogenic enzyme deficiencies. In particular, targeted LC-MS/MS steroid panels, besides being very well suited for the routine laboratory setting, have proven extremely useful in diagnosing CAH subtypes and guiding treatment. However, non-CAH PAI often remains without a definite cause in a substantial number of cases. Detailed clinical phenotyping of such cases is critically important for diagnostic workflow but genotyping is equally important, confirming the diagnosis or carrier state, providing prognostic information on disease severity and is essential for genetic counseling.

Adrenal insufficiency is associated with a reduced quality of life that may be caused by non-physiological glucocorticoid replacement. In recent years, a substantial amount of progress has been made in optimizing glucocorticoid delivery systems, as well as by exploring non-glucocorticoid therapeutic strategies in CAH. However, there is still a long way to go in developing disease-specific and personalized treatments for children with PAI.

Ethics

Peer-review: Internally peer-reviewed.

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REVIEW

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The Rationale for Growth Hormone Therapy in Children with Short Stature

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Abstract

Growth hormone (GH) was first isolated from cadaver pituitary glands, requiring laborious and expensive collection of glands, followed by extraction and purification of the hormone. This limited supply restricted its use to children with severe GH deficiency who were treated with low dosages and suboptimal schedules. The development of recombinant DNA-derived GH, allowed the production of virtually unlimited amounts of GH, leading to the approval for therapy for a large number of childhood conditions characterized by non-GH deficient short stature. The aim of this review is to provide a critical overview on the daily use of GH in two paradigmatic conditions of non-GH deficient short stature which are children born small for gestational age and with idiopathic short stature, highlighting the available strength of evidence for efficacy and safety.

Keywords: Growth hormone treatment, idiopathic short stature, small for gestational age

Introduction

Short stature is the most common cause of referral to pediatric endocrinology units, though the vast majority of short children have variants of growth such as constitutional delay of growth and puberty (CDGP) and familial short stature (FSS) (1,2,3,4).

Due to the shortage of human growth hormone (GH) prepared by extraction from pituitaries obtained at autopsy, for almost three decades GH therapy was limited to children with the diagnosis of GH deficiency (GHD) (5). Since 1985, when biosynthetic GH was first produced on a large-scale (6,7,8,9,10,11,12,13), the virtually unlimited availability led to a rapid expansion of clinical trials to study the effect of GH in various conditions associated with short stature but with normal GH secretion (14,15). One of the first conditions characterized by non-GH deficient short stature which was nevertheless treated with GH was Turner syndrome (TS) (16). The preliminary short-term trials, though reporting encouraging results, raised doubts about appropriateness and long-term effectiveness and safety (17). Other genetic syndromes such as Noonan syndrome (18) and achondroplasia (19,20) were considered as potential indications for GH therapy. Most of these pioneering studies with biosynthetic GH in non-GH deficient short children were short-term trials that considered the increase in height velocity after 6-12 months of GH therapy as the main outcome measure for assessing GH efficacy.

Following the publication of results from long-term trials, showing efficacy and safety of GH therapy, indications for such therapy have been expanded in the last two decades. Although the most frequent condition treated with GH still remains GHD, other growth-related indications for GH treatment are TS, short stature homeobox-containing (SHOX) gene deficiency, Noonan syndrome, Prader-Willi syndrome, growth failure associated with chronic renal insufficiency, short stature in children born small for gestational age (SGA) who do not demonstrate catch-up growth and idiopathic short stature (ISS) (Table 1).

Therefore, the initial GH replacement therapy limited to GH deficient patients has metamorphosed into a pharmacological therapy to include different conditions of non-GH deficient short stature. The rationale of this treatment is based on the empiric observation of growth acceleration in response to GH administration, rather than on a pathophysiological

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Table 1. Indications approved by Food and Drug Administration and European Medicines Agency for growth hormone therapy

Current indications for GH therapy	Regulatory authority
Idiopathic short stature	FDA
Familial short stature	
Non-familial short stature	
Primary growth failure	
Genetic syndromes	
Turner syndrome	FDA/EMA
SHOX deficit	FDA/EMA
Noonan syndrome	FDA
Prader-Willi syndrome	FDA/EMA
Silver-Russell syndrome	FDA/EMA
Other	
Small for gestational age	FDA/EMA
Secondary growth failure	
Growth hormone deficiency	FDA/EMA
Chronic systemic disease	
Chronic renal disease	FDA/EMA

GH: growth hormone, FDA: Food and Drug Administration, EMA: European Medicines Agency, SHOX: short stature homeobox-containing

approach. From a biological perspective, the close relation between GH dose and response to therapy, in terms of growth acceleration, is well established and confirms the clinical finding of excessive height gain in children with hypersecretion of GH-the more GH, the more growth.

The aim of this review is to provide a critical overview on the daily use of GH in two paradigmatic conditions of non-GH deficient short stature, namely SGA and ISS.

Small for Gestational Age

Children born SGA are at risk of becoming short adults. Although most children born SGA show catch-up growth in the first 24 months of life, approximately 10% remain below the 3rd centile throughout childhood and adolescence and into adulthood (21). To date, however, the mechanisms underlying postnatal catch-up growth in children born SGA are still largely unknown (22). Birth length is a more important predictor of adult height than birth weight (22,23,24,25) and though genetics play a key role in controlling the growth trajectory, the endocrine mechanisms underlying early growth remain undetermined.

SGA refers to the size at birth and is defined as a birth weight and/or length of at least two standard deviation (SD) scores (SDS) below the mean for gestational age and gender (26,27). The etiology of intrauterine growth retardation ultimately leading to SGA consists of a broad spectrum of maternal, environmental, placental and fetal factors, but in a significant proportion of cases the reason for being born SGA remains unclear.

SGA newborns show high circulating levels of GH and low concentrations of both insulin-like growth factor 1 (IGF-1) and IGF-binding-protein-3 which normalize in the first months of postnatal life, thus suggesting a transient GH insensitivity (28,29). In childhood and adolescence, SGA subjects show normal GH responses to stimulation tests (30). Alterations in diurnal GH secretion profile have been reported by isolated studies but are of limited diagnostic and prognostic utility (31,32). On average, both IGF-1 and IGF-binding protein-3 levels are reduced in SGA children by approximately one SD, but the individual variability is wide, indicating broad heterogeneity in the underlying endocrine and non-endocrine mechanisms.

Genetic abnormalities in the GH-IGF axis such as IGF-1 and IGF-1 receptor gene deletions and point mutations have been associated with small size at birth and severe postnatal growth retardation (33,34,35).

The first short term trials with pituitary derived GH in short SGA children date back more than 50 years (36,37). More recently, a promising short-term trial with biosynthetic GH (38) paved the way for long-term studies whose results led to the approval from regulatory authorities such as the Food and Drug Administration (FDA) in 2001 and European Medicines Agency (EMA) in 2003, although with slightly different criteria. FDA approval includes a dose of 0.48 mg/kg per week for treatment of children born SGA who fail to manifest catch-up growth by the age of two years, whereas EMA approved GH for the treatment of short children born SGA after the age of four years at a dose of 0.22 mg/kg per week.

A consensus conference organized by the main international societies of Pediatric Endocrinology and the Growth Hormone Research Society proposed that children born SGA with height less than minus 2.5 SDS at the age of two years or with height less than minus 2 SDS at the age of four years should be eligible for GH treatment. The dose should range from 35 to 70 μg/kg per day, with the higher dose to be preferred for those with more severe growth retardation (30).

The improvement of adult height is unanimously considered the best outcome measure of the efficacy of GH therapy in SGA. The approval of this indication was based on the results from a few randomized controlled trials (RCTs) conducted until the achievement of adult height. Moreover, the available data were collected from small study cohorts treated with different treatment regimens. We set out to critically evaluate the strength of evidence by performing

a systematic review and meta-analysis of all the available trials (39). The results of this meta-analysis showed that from an initial number of 29 studies reporting the effect of GH therapy in SGA children, only four RCTs were conducted up to the achievement of adult height, and these four studies included a total of 391 children (40,41,42,43). The mean adult height of the GH-treated group exceeded controls by 0.85 SDS (5.7 cm) after eight years of therapy. Furthermore, no significant difference in efficacy was observed between the two GH dose regimens (33 vs. 67 μg/kg per day) (Figure 1). A wide individual variability in response to GH therapy was present in all studies, consistent with the heterogeneity of conditions underlying SGA. The quality grading of the studies was performed according to Endocrine Society criteria (44) and revealed that all four RCTs had moderate quality evidence.

Although there is a large body of evidence suggesting that low birth weight is associated with a high risk of developing insulin resistance, glucose intolerance and metabolic disorders in later life, thus far GH treatment in SGA children has not been associated with major side effects. A transient insulin resistance, increased fasting glucose and reduced tolerance during oral glucose-tolerance testing have been reported (42,43,45,46). Longer follow-up of SGA subjects treated with GH during childhood, up to six years after discontinuation of therapy, showed a similar body composition, insulin sensitivity, blood pressure and a more beneficial lipid profile compared with untreated, short, young adults born SGA (47,48,49).

GH treatment has been reported not to influence the age at onset and progression of puberty, regardless of the dose (50) and duration of puberty and pubertal height gain are apparently not affected by the use of higher doses of GH (50,51,52). Moreover, GH therapy seems to improve body composition and cardiovascular profiles in children born SGA, reducing fat mass, blood pressure and lipid levels and increasing lean body mass (46,53). Even intelligence, psychosocial functioning and quality of life (QOL) have been reported to improve during GH therapy in SGA children (54,55,56).

	Treated children			Control children				
RCTs	Mean	SD score	Total	Mean	SD score	Total	Weight %	Mean difference (95% IC)
Carel et al (40)	-2.1	1	102	-2.7	0.9	47	21.3%	0.6 (0.28-0.92)
Dahlgren et al (41) <2 anni	-1.6	0.8	41	-2	0.8	34	20.2%	0.40 (0.04-0.76)
Dahlgren et al (41) >2 anni	-1.2	0.7	36	-2	0.8	34	20.5%	0.8 (0.45-1.15)
van Dijk et al (43)	-1.4	1	37	-2.6	0.6	25	19.2%	1.20 (0.8-1.60)
Van Pareren et al (42)	-1	0.8	54	-2.3	0.7	15	18.8%	1.30 (0.89-1.71)
Totale (95% IC)			270			155	100%	0.85 (0.52-1.17)

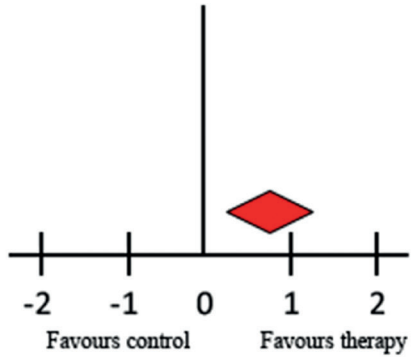

Figure 1. Effect of long term growth hormone therapy on adult height in randomised controlled trials. Results of meta-analysis according to random model (39) in children born small for gestational age. The mean difference in adult height between treated and untreated children was 0.85 standard deviation (IC 95% 0.52-1.17, $p < 0.001$)

SD: standard deviation, RCTs: randomized controlled trials

In general, GH therapy is not indicated in SGA during adolescence due to the reduced growth potential remaining after entering puberty. However, combined therapy with GH and gonadotropin releasing hormone analogs (administered for two years) has recently been reported to be safe and effective in improving adult height in SGA children with more severe growth retardation at the onset of puberty (57,58).

Children with Silver-Russell syndrome (SRS) constitute a syndromic subgroup of SGA and were classically considered to be less, or even non-responsive, to GH therapy (30). Smeets et al (59) have recently reported that SRS children are significantly shorter than non-SRS SGA children at start of GH therapy but gain more height during treatment, resulting in a similar height SDS at onset of puberty in SRS and non-SRS. Thereafter, there is a decline in height SDS from puberty onset to adult height attainment in SRS compared to non-SRS, leading to a significantly shorter adult height (59). However, although SRS children do not attain the same adult height as non-SRS, the total height gain is similar suggesting a positive growth promoting effect of such therapy (59). In addition, a positive effect of GH therapy on body composition, motor development, appetite and reduced risk of hypoglycemia has been reported (59,60).

Wide individual variability in response to GH therapy has been reported in all studies. Multiple linear regression analyses were used to construct the best model for predicting adult height SDS. The major predictors of adult height reported so far are: (i) height and weight at the start of GH treatment; (ii) target height; (iii) pretreatment growth rate; and (iv) prepubertal years treated with GH (61,62).

Is GH therapy a panacea for children born SGA? Although the results of most studies strongly encourage GH treatment in SGA children, it has to be pointed out that (i) the longitudinal follow-up is still relatively short; (ii) the overall number of children enrolled in the trials is relatively small, but, more importantly, (iii) almost all studies have been performed by the same group of investigators in the Netherlands, thus leaving open the question about the replicability of their results in other geographical and scientific contexts.

Idiopathic Short Stature

The story of GH treatment in children with ISS begins in 1983 with the first trial conducted with pituitary derived GH in 15 non-GH deficient short children who were treated for six months (15). In all children, growth rate increased by more than 2.0 cm per year during treatment. This short-term study paved the way for a series of trials which led to FDA approval for such an indication in 2003.

ISS is defined as a condition in which the height of an individual is more than 2 SDS below the corresponding mean height for a given age, sex and population, without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities (63,64). Therefore, children defined as having ISS have a normal size at birth and normal GH secretion. ISS is defined by criteria rather than a diagnosis per se and encompasses a variety of conditions including both mild skeletal abnormalities not falling into any of the known, classified disorders and non-syndromic genetic conditions, as well as normal variants of growth such as CDGP and FSS (65).

In 2003, GH therapy was approved in the United States for children with ISS with height at or less than -2.25 SDS (1.2 percentile) below the mean for age and sex, associated with growth rates unlikely to permit attainment of adult height in the normal range, and in whom diagnostic work up excluded other causes for short stature that should be observed or treated by other means. A consensus conference of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society proposed that children with ISS whose heights are less than -2.0 SDS and who are more than 2.0 SDS below their mid-parental target height or had a predicted height less than -2.0 SDS warrant consideration for treatment (63).

However, controversy still exists about the degree of effectiveness of GH therapy in children with ISS (66). A preliminary systematic review of literature showed that one year of GH therapy induced an acceleration of growth velocity and suggested that long-term GH therapy was able to increase adult height (67). However, this systematic review did not consider the outcome measures analytically and did not evaluate and classify the trials according to the quality of evidence and strength of recommendation. Furthermore, at that time, the review could take into account the results of only one randomized control trial, limited to eight girls followed up to the achievement of adult height (68). The authors, cautiously and wisely concluded that the focus of assessment should increasingly shift from efficacy in promoting growth to effectiveness in promoting health and well-being as a function of increased growth (67).

A more detailed and updated meta-analysis of available trials, including quality grading according to the Endocrine Society criteria which classifies the quality of evidence into one of four categories (high, moderate, low and very low) (44) was performed by the authors of this review in 2011 (69). The aim was to systematically determine the impact of GH therapy on adult height of children with ISS. This systematic review of the literature showed that from an initial number of 19 long-term trials, only ten met

the criteria of controlled trials. Three RCTs (including 115 children, 79 cases and 36 controls) (68,70,71) and seven non-RCTs (including 477 children, 181 cases and 296 controls) (72,73,74,75,76,77,78) reported data on adult height. Two randomized clinical trials were classed as of moderate quality evidence and one of low quality evidence. Six non-randomized clinical trials were classed as of low quality evidence and one of low-moderate quality evidence. The adult height of the GH treated children exceeded, on average, that of the controls by 0.65 SD (about 4 cm) (Figure 2). In the seven non-RCTs, the adult height of the GH treated group exceeded, on average, that of the controls by 0.45 SDS (about 3 cm).

The main conclusions were that: (i) no single, high quality evidence, RCT was carried out up to the achievement of adult height; (ii) that the overall magnitude of GH effect in reducing the adult height deficit in children with ISS was on average less than that achieved in other conditions for which GH was licensed and; (iii) that the individual response to therapy was highly variable (69).

More recently, van Gool et al (79) have reported that high dose GH therapy in prepubertal children with ISS does not improve adult height, as it increases height gain during treatment but, at the same time, accelerates bone maturation, resulting in a similar adult height compared with the untreated controls.

Finally, the effect on adult height of a combined therapy with GH plus gonadotropin-releasing hormone analogs in ISS adolescents with relatively early puberty was assessed. The modest results in height gain led the authors to advise physicians against the use of this treatment in clinical practice (80).

Because estrogens mediate skeletal maturation and epiphyseal fusion, aromatase inhibitors have been used to delay bone maturation. The first trial with aromatase inhibitors was performed in boys with CDPG with apparently promising results (81,82) and, afterwards in children with ISS alone (83) or in combination with GH (84,85). These still preliminary results indicate that aromatase inhibitors, especially in combination with GH, seem to be effective

	Treated children			Control children				
RCTs	**Mean**	**SD score**	**Total**	**Mean**	**SD score**	**Total**	**Weight %**	**Mean difference (95% IC)**
Albertsson-Wikland et al (71) 0.033 mg/kg/day dose	-1.70	0.68	18	-2.20	0.75	19	30.0	0.50 (0.04-0.96)
Albertsson-Wikland et al (71) 0.067 mg/kg/day dose	-1.50	0.84	31	-2.20	0.75	19	31.7	0.70 (0.25-1.15)
Leschek et al (70)	-1.77	0.80	22	-2.34	0.56	11	28.8	0.57 (0.10-1.04)
McCaughey et al (68)	-1.14	1.06	8	-2.37	0.46	6	9.4	1.23 (0.41-2.05)
Totale (95% IC)			79			55	100.0	0.65 (0.40-0.91)

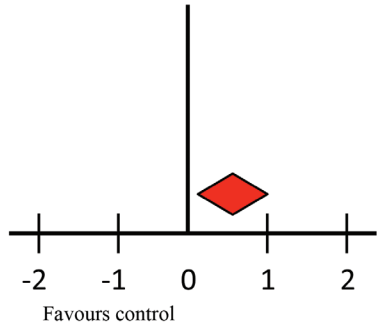

Figure 2. Effect of long term growth hormone therapy on adult height in randomised controlled trials. Results of meta-analysis according to random model (69) in children with idiopathic short stature (ISS). The mean difference in adult height between treated and untreated ISS children was 0.65 standard deviation (IC 95% 0.4-0.91, $p < 0.001$)

SD: standard deviation, RCT: randomized controlled trial

in stimulating growth. However, caution is needed as potential adverse effects include reduced high-density lipoprotein cholesterol, increased insulin resistance, vertebral deformities, impairment of cognitive function and long-term effects on spermatogenesis and infertility (86). Therefore, the use of aromatase inhibitors must be considered experimental and to be performed only in strictly controlled clinical trials.

Wide individual variability in the response to GH therapy was reported in all clinical trials conducted in ISS children. The major predictors of adult height reported so far were: (i) early age at start of therapy; (ii) dose of GH; (iii) length at birth; (iv) difference between height and mid-parental height and; (v) delay in bone age (87).

In conclusion, the available evidence suggests that long-term GH therapy reduces the adult height deficit in children with ISS. The still open question is whether this treatment is worthwhile considering the impact of the height gained on physical and psychosocial wellbeing, burden for patients and parents, potential adverse effects, cost of therapy and patients'/parents' expectations.

Final Remarks

The available evidence shows that GH therapy can increase adult height in non-GH deficient short children born SGA or with ISS. However, in both conditions the efficacy is far less than in GHD. A critical review of available data shows that to date, no study has fulfilled the evidence based medicine criteria for high quality of evidence and strong recommendation. The individual response to therapy is highly variable and further studies are needed to identify what defines the responders.

The assumption underlying the pharmacological use of GH in non-GH deficient short children is that GH treatment, by increasing adult height, improves the QOL of subjects with short stature. However, data are conflicting and inconclusive, and this potential effect cannot be considered at the moment as a strong argument for such therapy (55, 88,89,90,91,92,93,94,95,96).

The long-term safety of GH therapy has recently been questioned by observational studies reporting increased risk of mortality and morbidity in young adults treated with GH during childhood (97,98). Although these data have not been confirmed (99,100,101), continued surveillance of subjects exposed to recombinant human GH is essential both during treatment and in the years after treatment cessation (102,103).

Finally, further high-quality evidence from randomised, double blind, placebo controlled trials up to the achievement of adult height would be necessary to determine the real efficacy, ideal dosage and long term safety of GH therapy in non-GH deficient short children.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Stefano Cianfarani, Annalisa Deodati, Design: Stefano Cianfarani, Annalisa Deodati, Analysis or Interpretation: Stefano Cianfarani, Annalisa Deodati, Literature Search: Stefano Cianfarani, Annalisa Deodati, Writing: Stefano Cianfarani, Annalisa Deodati.

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REVIEW

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A Critical Appraisal of the Effect of Gonadotropin-Releasing Hormon Analog Treatment on Adult Height of Girls with Central Precocious Puberty

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Abstract

Central precocious puberty (CPP) is a diagnosis that pediatric endocrinologists worldwide increasingly make in girls of age 6-8 years and is mostly idiopathic. Part of the reason for increasing referral and diagnosis is the perception among the doctors as well as the patients that treatment of CPP with long-acting gonadotropin-releasing hormon analogues (GnRHa) promote height of the child. Although, the timing and the tempo of puberty does influence statural growth and achieved adult height, the extent of this effect is variable depending on several factors and is modest in most cases. Studies investigating GnRHa treatment in girls with idiopathic CPP demonstrate that treatment is able to restore adult height compromised by precocious puberty. However, reports on untreated girls with precocious puberty demonstrate that some of these girls achieve their target height without treatment as well, thus, blurring the net effect of GnRHa treatment on height in girls with CPP. Clinical studies on treatment of girls with idiopathic CPP on adult stature suffers from the solid evidence-base due mainly to the lack of well-designed randomized controlled studies and our insufficiencies of predicting adult height of a child with narrow precision. This is particularly true for girls in whom age of pubertal onset is close to physiological age of puberty, which are the majority of cases treated with GnRHa nowadays. Heterogeneous nature of pubertal tempo (progressive vs. nonprogressive) leading to different height outcomes also complicates the interpretation of the results in both treated and untreated cases. This review will attemp to summarize and critically appraise available data in the field.

Keywords: Central precocious puberty, gonadotropin-releasing hormon analogues, treatment, final height, adult height, growth, triptoreli, leuprolide

Introduction

Gonadotropin-releasing hormone analogues (GnRHa) are the treatment of choice for nearly four decades in children with central precocious puberty (CPP) (1). Treatment effectively suppresses hypothalamo-pituitary-gonadal axis, which results in arresting early and accelerated activation of sex hormone synthesis, progression of secondary sexual characteristics and undue maturation of the skeletal development, thus meeting the aims of the treatment, which are 1) to prevent potential psycological problems related to early pubertal development, and 2) to restore genetic growth potential otherwise compromised by sex-hormone-driven premature closure of bone growth plates.

Although the majority of the studies in the field suggests beneficial effects of treatment, there have been ongoing uncertainties about the achievement of both aims of the treatment due to methodological limitations of the present studies. This review wil focus on the effect of GnRHa treatment on height outcome in girls with CPP.

Uncertainties about the benefits of GnRH analog treatment on growth of children with CPP comes from the fact that there is no randomised controlled study on this respect. Some of the studies in this field compare treatment group with a historical control groups which are reported decades ago, include limited number of subjects and heterogeneous with respect to nuances of pubertal development. Some studies have their own untreated control group (but not

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randomised) which brings biases to the interpretation of the data. Finally, many studies are comparing the achieved adult height with predicted adult height (PAH) at initiation of treatment which suffers from the limitations of our ability to assess bone age (BA) and predict adult height precisely, and disregard the genetic height potential of the child.

The Relationship Between Height and Timing of Puberty

It has been known for a long time that physiological variations in the time of pubertal development has an effect on statural height. Shangold et al (2) evaluated the relationship between recalled menarcheal age and adult height, in 425 women. After exclusion of those in whom menarche occurred after age 16, the overall linear regression equation for the remaining 416 patients, height = 153.95 + 0 .7353 x (age of menarche), was significant. Average height in women who had menarche at age 9 was 159.5 ± 6.5 whereas those with menarche at age 11-13 yrs was 163 cm. Overall the data suggested that menarcheal age significantly correlates with adult height as an independent variable (2).

A large longitudinal study on American girls also evaluated the effect of timing of spontaneous puberty on height was indicated a higher adult height in girls with late (> 12.9 years) versus early (< 11.7 years) age at menarche. The median difference was of 2.6 and 1.7 cm in white and black girls respectively (3). A recent Korean study of 4218 post-menarcheal girls between the ages of 16 and 18 years reported mean heights of early (9.9 ± 0.2 years), average (12.5 ± 0.9 years) and late (15.1 ± 0.3 years) menarche groups as 160.4 ± 5.2 cm, 161.8 ± 4.9 cm, 162.3 ± 4.7 cm respectively p = 0.001) (4).

In contrast to above studies, a recent longitudinal study from Thailand followed 104 girls with breast development at 7.0-9.0 years. Despite the average age at menarche was early (10.2 ± 0.9), their near final height obtained at 12.6 ± 0.4 years was 154.0 ± 4.9 cm, which was similar to their average target height (TH) of 153.1 ± 4.8 cm (5).

It can be concluded from above mentioned studies that "early" puberty within the currently accepted physiological range has "if any" a very small (2-4 cm) effect on adult height reached, an observation consistent with none to very small height gain achieved in GnRH analog treatment of girls with "early" puberty (6,7,8). However, "truely" precocious puberty starting at a very young age is expected to result in more loss in height potential depending on the age at start and the tempo of puberty. Precise estimation of the height loss caused by precocious puberty is difficult to estimate because of the scarcity and imperfections of data in that respect.

Height Outcome in Girls with Precocious Puberty without Treatment

Historical series of untreated patients (Table 1) reported mean heights of 152 cm in girls and 156 cm in boys, a loss of ~10 cm in girls and 20 cm in boys (9,10,11,12,13,14). However, these data should be interpreted very cautiously. First of all, those data come from a limited number of patients from the 1950s and 1960s with cases that are very severe and early onset CPP, with cases due to organic reasons constituting the great part of it. Thus, more severe than the average patient treated today. In fact, in most of these historical series, there was a negative correlation between the age of onset of precocious puberty and adult height, confirming the poor height prognosis of the most severe and early cases. Furthermore, some of the untreated patients with organic CPP may have had growth limitation due to factors associated with their central nervous system disorder, such as growth hormone (GH) deficiency. Secondly, these were not large series, especially for the figure of boys which derived from total of 38 untreated boys in total of four studies (9,10,11,12). Lastly, these studies do not take into account the secular increase in height.

In one of the early studies, Paul et al (11) compared their treated patients with untreated subjects from the literature (9,10,13,14). The final height of treated females was 160.5 ± 6.6 cm whereas matched untreated historical females had a height of 152.7 ± 8.6 cm (difference of treated vs. untreated 7.8 cm). Although treated girls' mean final height was still -1 standard deviation (SD) below mean midparental TH, this was better compared to untreated ones who had height -2.4 SD below TH. Further classification of

Table 1. Historical data of untreated children with precocious puberty

Reference	No. of patients (female/male)	Mean final height ± SD (cm)	
		Females	Males
Thamdrup (9)	26/18	151.3 ± 8.8	155.4 ± 8.3
Sigurjonsdottir and Hayles (10)	40/11	152.7 ± 8.0	156.0 ± 7.3
Paul et al (11)	8/4	153.8 ± 6.8	159.6 ± 8.7
Bovier-Lapierre et al (12)	4/5	150 ± 6.2	156 ± 6.3
Lee (14)	15/0	155.3 ± 9.6	-
Werder et al (13)	4/0	150.9 ± 5.0	-

Total: 107 F/38 M

SD: standard deviation

the patients according to age revealed that untreated girls who were <5 years of age had a mean final/near final height of 150.2±7.6 cm whereas those treated reached 164.3±7.7 (difference of treated vs. untreated 14.1 cm). Untreated girls who were >5 years of age had a mean final height of 153.4±8.4 cm whereas those treated reached 157.6±6.6 (difference of treated vs. untreated 4.2 cm).

Kletter and Kelch (15) reviewed this matter in 1994. They found more modest height gains in treated girls compared to untreated girls (6.5 cm and 0.5 cm in <6 yrs and >6 yrs respectively). However, when they compared patients with their TH, the effect of GnRHa treatment on height was much less (only 2.7 cm in whom puberty started before 6 years of age and no height gain in those >6 yrs). The authors concluded that treatment with GnRH agonist analog does not significantly alter the final adult height of girls with idiopathic CPP whose age at diagnosis is greater than 6 years.

The obvious difference between the conclusion of these studies might arise from the heterogeneity of the subjects in regard to TH, and the tempo of puberty in the subjects (both treated and untreated). As most untreated patients in these series were seen before the introduction of computed tomography it is quite possible that some who had a small intracranial lesion, for example a small hypothalamic hamartoma, were included in this untreated "seemingly" idiopathic CPP groups.

Nevertheless, those studies with untreated control groups (Table 2) (11,15), as well as later studies without control groups (Table 3) (16,17), confirmed that age is an important determinant of treatment outcome and that earlier the age of onset of CPP, the worst is the height outcome if left untreated. Thus, earlier the onset of treatment, height gain achieved by the GnRHa treatment is bigger.

However, unlike historical untreated cohorts mentioned above, some studies afterwards reported final height of untreated girls with CPP demonstrated less, or no decrease in height compared to their TH. Bar et al (18) reported final height data of 20 and near final of 7, girls with idipathic CPP. The appearance of breast tissue occurred at 5.6±1.6 years; the first evaluation was performed at 7.0±2.4 years. Six children were less than 6 years of age at the time of the initial evaluation. Although the mean BA was 8.4±3 years, one third of the girls had a BA at least 2 years (range, 2 to 3.7

Table 2. Adult heights (cm) of treated and untreated (historical) girls with central precocious puberty according to the age of onset

Paul et al (11)		Untreated <5 yr (n=41)	Treated <5 yr (n=11)	Untreated >5 yr (n=75)	Treated >5 yr (n=15)
	Final height	150.2±7.6	164.3±7.7	153.4±8.4	157.6±6.6
		Height difference: 14.1 cm		Height difference: 4.2 cm	
Kletter and Kelch (15)		Untreated <6 yr (n=10)	Treated <6 yr (n=17)	Untreated >6 yr (n=54)	Treated >6 yr (n=114)
	FH	153.9±3.8	160.4±1.8	157.0±0.9	157.5±0.6
		Height difference: 6.5 cm		Height difference: 0.5 cm	
	TH	160.7±1.7	164.5±1.4	159.0±0.9	161.4±0.6
	FH-TH	-6.8 cm	-4.1 cm	-2 cm	-3.9 cm
		Net height gain from treatment: 2.7 cm		Net height gain from treatment: -1.9 cm	

FH: final height, TH: target height

Table 3. Effect of age of onset of treatment on height (studies with no control group)

	CA at onset		BA at onset		<6 yr			>6 yr		
	<6 yr	>6 yr	<6 yr	>6 yr	PAH	TH	FH	PAH	TH	FH
Partsch et al (16)	5.0±0.4	7.8±0.2	8.4±0.5	10.4±0.3	152.1±2.2	162.4± 1.08	161.6±1.43	157.7±1.8	165.3±1.43	159.4±1.75
Lazar et al (17)	6.4±1.2	7.5±0.6	11.3±0.4	11.3±0.3	154.6±6.6	159.3±5.0	162.8±5.0	157.8±5.2	153.7±6.7	157.9±5.1

CA: chronological age, BA: bone age

years) greater than their chronologic age. The mean age of menarche was 10.5 years which was 4.9 ± 2.4 years (range, 3 to 13 years) after thelarche. Despite that, adult height was normal in 90% of girls (mean, 161.4 ± 7.7 cm). Although parental heights were not available in this study, mean final height of the untreated girls with ICCP were only slightly less than healthy average American women 163.8 cm.

In another study, untreated control group consisted of 10 girls with idiopathic precocious puberty who, at their parents' request, were not treated. Mean age at the onset of pubertal signs was 6.05 ± 0.3 years. There was no significant difference between final height of treated (152.4 ± 1.4 cm) and untreated (149.5 ± 2.0 cm) girls. Final height was significantly lower than TH in both treated (with ciproteron) (155.1 ± 0.9 cm; and untreated (156.4 ± 1.3 patients, but the mean height of treated patients is nearer to TH than that of untreated ones (19).

In a similar study, Kauli et al (20) reported final height of 28 untreated girls with ICCP. Fourteen of them had a slow course of puberty and reached final height of 160.2 ± 7.1 (their TH was 159.5 ± 6.6 cm); the other half (14/28) had an accelerated course of puberty with a final height well below TH (final height 150.8 ± 4.3, TH 159.2 ± 5.9 cm) and in most cases (14/28) below the height SD score (SDS) of both parents.

Obvious differences in the height outcome of untreated patients in different studies (historical cohorts versus more recent cohorts) reflect the heterogeneity of the patients in regard to pubertal hormonal activation. As in Kauli et al's (20) study, it has been shown in several series that in a subgoup of the girls presenting with what appears to be idiopathic CPP, will either have stabilization or very slow progression in their pubertal signs. Progression of hormonal activation is somewhat slower in these girls and the final heights are not compromised. The BA is typically not as advanced compared with children with true CPP, and serum lutenizing hormone (LH) concentrations are within the pre- or early-pubertal range, indicating that the hypothalamic-pituitary-gonadal axis is not fully activated. GnRH stimulation test in these children demonstrate a follicle-stimulating hormone (FSH) dominant response. These children are considered to have slowly progressive form of CPP.

Palmert et al (21) reported 12-yr follow-up of 20 patients who initially presented with unsustained or slowly progressive puberty by the presence of one or more of the following findings: menses, pubic hair, accelerated growth velocity, and/or BA greater than 2 SD above chronological age. None of the 20 patients had a pubertal response to exogenous GnRH; (by that time with an radioimmunoassay LH increase of less than 25 IU/L above baseline and a peak FSH greater than or equal to the peak LH in response to exogenous GnRH). Thus, at that time, these girls were not considered candidates for long term pituitary-gonadal suppression with a GnRH agonist. Seventy percent of those patients experienced cessation of their early pubertal development, whereas the remainder reported a slowly progressive course. Those with a slowly progressive course were significantly older than those with an unsustained course [mean age of thelarche, 6.1 vs. 3.4 yr; age of pubarche, 6.0 vs. 4.0 yr. They also had more advanced skeletal maturation (BA, 10.2 vs. 7.3 yr; at the time of evaluation. Both groups, however, had similar outcomes with respect to linear growth and young adult reproductive function. On the average, the study patients reached their genetic targets for final height (mean final height, 165.5 ± 2.2 cm; mean genetic TH, 164.0 ± 1.1 cm; p 5 NS). The average age of menarche was 11.0 ± 0.4 yr.

Léger et al (22) also followed 9 patients (mean age 6.5 years, range 4.8-7.7 years) with a slowly progressing variant of CPP without treatment; final height (161.8 ± 4.6 cm) was similar to the pre-treatment predicted height (163.1 ± -6.2 cm) and was not significantly different from TH (161.0 ± 5.9 cm).

Table 4 summarizes height outcome of girls with untreated CPP (slowly progressive, milder, or older onset) patients in different series. Final height-TH ranged between -6.8 cm to 1.6 cm. On average, final height was -4.4 cm shorter than TH in six studies (15,18,19,20,23,24) but similar to TH in the remaining seven studies (20,21,22,25,26,27,28). Thus, it can be concluded that the different height outcome of girls with untreated idiopathic CPP in various studies are due to the fact that natural course of precocious puberty differs from one subject to another, i.e. some are more progressive hence have unfavorable outcome whereas some are slowly progressive hence favorable outcome in regards to final height.

Identification of Girls with Progressive Central Precocious Puberty

There is not enough data about the ratio of progressive vs. nonprogressive precocious puberty among girls who develop breast development before 8 years of age. Kaplowitz (29) reported 9% of true precocious puberty in 104 children referred for any signs of early puberty, whereas this ratio was higher (47%) in another US study of 223 girls referred for precocious puberty between ages 7 and 8 (white girls) or 6 and 8 (black girls) (30). Mogensen et al (31) reported nearly 20% true precocious puberty, among 449 girls referred for early pubertal signs. All of these cohorts included all variants of early pubertal development including premature thelarche, premature adrenarche and early normal variants (those > age 8 yrs). However, we have recently reviewed 236 girls who presented with breast development between ages

4-8 years (thus excluding premature adrenarche, thelarche variant etc.). 59% of these girls were eventually diagnosed with true precocious puberty and given GnRHa treatment (32). This was nearly 34% in Mogensen et al's (31) series after exclusion of other variants.

Although the mechanism of why puberty is nonprogressive in certain girls is unknown, some clinical features have been proposed to help identifying those who will likely to progress rapidly, although specifity and sensitivity of these criteria varies greatly (33,34,35,36,37,38,39,40) (Table 5). Along with clinical and anthropometric criteria, GnRH-stimulated LH levels of 5 IU/L have been suggested to mark the beginning of puberty using one modern immunochemiluminometric assays (34,35). Stimulated LH limit of 5 IU/L to define CPP was found to have specificity of (77%), and sensitivity of (95%) (36). In one study, randomly measured LH values of 0.3 IU per liter and above were reported to be 100% specific for peak values above 5 IU per liter (37). However, in young children (2-4 years) gonadotropin levels are normally high and therefore LH (basal or peak) should be carefully interpreted in this age group (38). In the consensus report on the use of GnRHa treatment, mentioned values for uterine length range from 3.4 to 4.0 cm (1). The cutoffs for a pubertal ovarian volume range between 1 and 3 mL (volume: length x width x height x 0.5233) (39). A uterine volume greater than 2.0 mL has been reported to have 89% sensitivity and specificity for precocious puberty (40).

As distinguishing progressive form of CPP from nonprogressive forms is important for therapeutic decision-making, the Consensus Conference Group has recommended that progressive pubertal development be documented for 3-6 months before starting GnRHa treatment This observational period may not be necessary if the child is at or past Tanner stage 3 (breast), particularly with advanced skeletal maturation (1).

In addition to above mentioned anthropometric and clinical criteria, we should be aware of certain risk groups in whom precocious puberty is likely to be progressive. These are, family history for precocious puberty, being born small for gestational age (SGA), and adopted children. One has to carefully follow these children when they develop breast development early, as they likely to have progressive precocious puberty. Familial forms of precocious puberty tend to be more progressive than those of sporadic ones. Comparison of 43 familial cases among the total cohort of 156 (147 girls and 9 boys) cases of idiopathic CPP, it was found that the familial group had lower maternal age at menarche than the sporadic group (mean, 11.47 +/- 1.96 vs. 12.66 +/- 1.18 yr; p = 0.0001) and more advanced puberty at admission (Tanner stage 2, 56.5% vs. 78.1%; p = 0.006). Segregation analysis suggested autosomal dominant transmission with incomplete, sex-dependent penetrance (41). Similarly reviewing case histories of familial CPP due to MKRN mutations reveal early and progressive nature of puberty in these girls (42).

Table 4. Final height of girls with untreated central precocious puberty (slowly progressive, milder, or older onset) patients in different series

	n	CA	BA	Age of menarche	TH	FH	Difference (FH-TH) cm
Bar et al (18)	20	5.6 (7.0)#	8.4	10.5	163.8*	161.4	-2.4
Kauli et al (20)	14^	-	-	-	159.5 ± 6.6	160.2 ± 7.1	0.7
	14				159.2 ± 5.9	150.8 ± 4.3	-8.4
Antoniazzi et al (23)	10	7.2 ± 0.9	9.6 ± 2.2	-	156.4 ± 1.3	149.6 ± 6.3	-6.8
Cisternino et al (19)	10	6.1	-	-	156.4	152.4	-4.0
Palmert et al (21)^	16	5.5	7.9	11	164.0	165.5	1.5
Brauner et al (25)^	15	7.9	9.4	10.4	161.0	162.0	1.0
Bertelloni et al (26)^	9	6.5			161.0	161.8	0.8
Léger et al (22)^	17	7.4	9.2	11.9	161.3	160.7	0.7
Allali et al (27)	52	8.0	9.1	-	161.4	163	1.6
Kletter and Kelch (15)	66	7.6 ± 0.24	10.1 ± 0.29	-	159.3 ± 1.1	156.5 ± 0.9	-2.8
Magiakou et al (28)	14	7.9	10.75	-	161.2	161.5	0.3
Balanli et al (24)	16	7.5 ± 2.0 (9.0 ± 2.1)#	10.9 ± 2.8	10.0	156.5 ± 5.2	154.5 ± 7.2	-2.0

#CA at the time of bone age determination is given in parenthesis

*Parental heights were not available. The height given is average healthy American women

^Patients were slowly progressing variants and or who had height prognosis above 155 cm thus treatment was not given

FH: final height, TH: target height, CA: chronological age, BA: bone age

Table 5. Criteria for identifying girls who are likely to have progressive precocious puberty

Progression of breast staging in less than 3-6 months
Growth velocity > 6 cm/year
Bone age advancement of more than 1.5-2 years
PAH below target height and decline in PAH during follow-up
Uterine volume > 2.0 mL, long diameter > 35 mm, presence of endometrial echo
Ovarian volume > 2-3 mL
Peak LH > 5.0 mIU/L at GnRH test, peak LH/FSH ratio > 0.66
Basal LH > 0.3 mIU/L, detectable basal E2

PAH: predicted adult height, LH: lutenizing hormone, GnRH: gonadotropin-releasing hormon, FSH: follicle-stimulating hormone, E2: estradiol

SGA-born girls are another special group of children in regard to puberty. Although being born SGA and having catch-up growth is clearly associated with premature pubarche and exagerated premature adrenarche, these children also have accelerated skeletal maturation and tend to have early (not necessarily precocious) but fast puberty resulting in short stature (43).

Finally the risk of developing precocious puberty was significantly increased in adopted girls and in these girls pubertal process usually continue progressively resulting in early menarche, rapid progression of BA and compromised adult height (44,45,46).

Bone Age-Based Treatment Decision

Some authors suggested predicted height-based decisions regarding GnRHa treatment of girls with CPP. Adan et al (47) used the criteria for treatment as; a PAH < 155 cm and/or a LH/FSH peaks ratio of > 0.6. Treatment group had greater breast development and BA advances (2.0 ± 0.2 years) and higher plasma estradiol concentrations than the group left untreated. Treated group achieved adult height of 159.5 cm, 3 cm taller than predicted height (156 cm), whereas untreated patients reached an adult height of 162,7 cm, 1.4 cm shorter than predicted height of 164.1 cm. Similarly, Léger et al (22) based treatment decision on BA and LH peak. They did not give treatment in those BA advancement is less than 2 years and peak LH < 6 mIU/mL at the initial evaluation. However they decided to begin treatment in girls whose PAH declined during treatment, and were able to achieve a final height better than PAH and surpassing the TH (22).

Thus, BA advancement, and as closely related to it, PAH have major determinants in decision making in regard to GnRHa treatment.

Handicaps in Bone Age Assessment

BA assessment is one of the key parameters in the management of patient with CPP as it allows the identification of rapidly progressing forms of CPP with compromised PAH, who are thought to benefit most from the treatment in respect to height. Periodical BA evaluation is also a part of monitoring treatment efficacy, as deceleration of BA maturation is expected as a result of treatment. However, BA assessment is affected by a great intra-observer variance, especially around BA of 8-10 years. Nowadays, the majority of girls who are being treated with GnRHa are those between the ages of 6 and 8 years with their BA in the range of 8-10 years.

Although there are several methods for evaluation of BA, the most commonly used method by pediatric endocrinologists is the Greulich-Pyle (GP) method. The GP method is an atlas method in which BA is evaluated by comparing the radiograph of the patient with the nearest standard radiograph in the atlas. Its simplicity, speed and precision made this method the most commonly used standard of reference for skeletal maturation worldwide. However, the GP method was developed using radiographs of upper-middle class Caucasian children in Cleveland, Ohio, United States, and the radiographs were obtained between 1931 and 1942 (48). One has to take the potential insufficiencies of this evaluation into account when evaluating children of today and children from various populations of different ethnic background. Furthermore, these BA methods are based on manual BA determination, the assessment is necessarily subjective and thus, have certain degree of inter-observer and intraobserver difference. In a study, three second year radiology registrars performed both Tanner-Whitehouse 2 (TW2) and GP assessments on each of the BA films. The average spread (the difference between the highest and the lowest of the three results) of BA readings was 0.74 years for TW2 method, and 0.96 years for the GP method (49). Bull et al (50) investigated 362 consecutive "BA" radiographs of the left hand and distal radius performed in a large provincial teaching hospital. Data were analysed using the "method comparison" statistical technique. Ten per cent of the radiographs were re-analysed to assess intra-observer variation. The 95% confidence interval for the difference between the two methods was 2.28 to -1.52 years. Intra-observer variation was greater for the GP method than for the TW2 method (95% confidence limit, -2.46 to 2.18 versus -1.41 to 1.43).

There is now, a recently developed an automated system of BA measurement using computerized image analysis based on both GP and TW2. The use of this automated system was validated in healthy children and in children with various endocrine disorders. It has been shown that automated systems have a better precision and accuracy compared to radiologists' reading (51). However, still, there

are differences in the interpretation of BA, which are big enough to influence clinical decision-making. In a recent study using an automated BA reading the BA difference between the most advanced and most retarded individual bones exceeded 2.0 years. The BA mean differences between the most advanced and most retarded individual bones were 2.58 and 2.25 years for the automated method and GP atlas methods, respectively (52).

Predicting Height in Girls with ICCP

Height prognosis of the child i.e. "PAH" is of major importance in clinical decision making in girls with CPP. Several alghoritms based on current height and BA to estimate adult height are available but none of them have been fully validated. Predicting adult height with accuracy is hampered by the problems in the accuracy of BA determination as well as problems of methodology in height prediction methods themselves. Bayley-Pinneau method is the most commonly used method for estimating adult height in children have been validated for height prediction in normal children (53). Bayley-Pinneau method estimates adult height as a percentage of current height, based on BA and its relationship to chronological age. It has a wide 95% confidence interval of about 6 cm below to 6 cm above the predicted value, a range which is large enough to mask or blunt small losses or gains in height that occurs due to precocious puberty or its treatment. The prediction equation differs for children whose BA is similar, retarded or advanced in comparison to chronological age (retarded, average and advanced columns in the Bayley-Pinneau height prediction table). Since children with precocious puberty has advanced BA, "advanced column" is used to predict height in girls with CPP. However, it has been shown in several studies that in untreated girls with precocious puberty, Bayley-Pinneau method tend to overrestimate final height by 3.7-5.9 cm in different studies (18,20,23).

To overcome this systematic error it has been proposed that "average column" should be used instead of advanced column (20). This approach might correct the systematic error but is unlikely to increase the precision. Studies reporting PAH in GnRHa treated girls by both advanced and average column demonstrates that final height is closer to PAH calculated with the advanced column than that of the average column (20,26,28,54,55,56). A recent study, when PAH was calculated using the average standards of GP, the median delta final height-PAH was 6.96 and 3.34 cm in GnRHa-treated and nontreated subjects, respectively, whereas when the accelerated standards were used, the differences were less (1.7 and 1.2 cm, p:NS). Final height-PAH-average and final height-PAH-accelerated were comparable among the nontreated subjects but among GnRHa-treated subjects, final height-PAH-average was significantly higher than final height-PAH-accelerated (28). Thus it appears that using advanced column for height prediction gives a better estimation of final height to be reached.

Height Outcome in Studies with Gonadotropin-Releasing Hormone Analogues Treatment of Progressive Central Precocious Puberty (Table 6)

GnRHa treatment has been a standart of care in girls with progressive CPP for nearly four decades now. GnRHa treatment decreases gonadotrophins, estradiol and the growth velocity and decelerates the skeletal advancement. Linear growth gradually decrease to a rate which is normal for a prepubertal child (~5 cm/year) during the first or second year of treatment, sometimes further deceleration happens in the following years (57,58). Bone maturation also slows down beginning from the 6 months of treatment, averaging 0.5+0.2 BA year/year (59). Similar values have been recorded in other reports (60,61,62). This decrease in bone maturation is progressive and does not occur before six months of treatment (63). As a result of the progressive normalization of BA, and continued linear growth, treatment provides increase in PAH despite the decreased growth velocity, although it is difficult to predict precisely the effect of GnRHa treatment on height gain of these patients, due to handicaps discussed above. Reviewing the available 28 studies on GnRHa treatment of CPP (7,15,16,17,20,22,23, 28,47,54,55,56,59,60,61,62,63,64,65,66,67,68,69,70,71, 72,73,74) (Table 6) and our own experience (75) demonstrate that the mean age at onset of pubertal development ranged 3-8 years, usually younger and more severe cases in older studies, older and milder cases in recent studies. Nevertheless, in most series, the age of treatment initiation was around 7 years, (5.4-8.7 years) with again recent studies tend to be a year later around 7.5-8 years. Mean BA was around 10 years (8.9-12.5 years) at the beginning of treatment and most series report mean treatment durations around 3.0 years. Mean chronological age at the end of treatment was around 11.1 (9.4-12.7) years of age with a mean BA of 12.4 (11.9-13.6) years. At the achievement of final height all studies except two (69,73) reported final height better than PAH (ranging 2.0-10.5 cm). On average, final height was ~4.0 cm higher than height predicted at the time of initiation of GnRHa treatment.

Comparison of final height of treated patients with their TH eliminates the handicaps of predicting adult heigt thus allows perhaps a better estimation of the effect of GnRHa treatment on height. When compared to TH, in most studies (nineteen studies), final height was 0.4 to 5.2 cm shorter than TH (15,16,20,28,47,54,60,61,62,63,64,65,66,67, 68,69,70,71,74) but 0.4-4.2 cm taller in the remaining nine

studies (17,22,23,55,56,59,72,73,75). On average, final height was ~1 cm shorter than TH.

However, one should also be aware that, even with comparison with TH is not free of biases. Calculation of midparental TH assumes equal contribution of each parents heights to the offsprings height, thus neglects the effect of dominant genes from one parent. Although TH correlates well with the offsprings height on a population level, it may not correlate well with individual subject. This is especially true for children whose parents are discordant for height.

Finally, in a limited number of studies when adult height of treated patients were compared with untreated study subjects, mean difference ranged from -3.0 to +11 cm) (20,23,28,75). Again, height gain was highly variable among studies depending on sample characteristics including the progression of pubertal development. It should be bear in mind, that the treatment effect also might be overestimated since most of the studies describe observed cases and none of them comprise an intention-to-treat analysis. It is possible that the patients who interrupt the treatment early and are not followed to adult height might have a poorer height outcome than those who continued to the end. Finally, predicted height values obtained during treatment are often overestimated in comparison to the adult height eventually achieved by the patient (7,33).

Factors Influencing Height Outcome

As mentioned earlier, and seen in the Table 2 data of historical untreated girls with CPP demonstrated that earlier the age of onset of puberty, worse the height prognosis. In line with that, evaluation of treatment series also show that younger age of onset of CPP and hence, younger age of initiation of treatment (which also means longer duration of treatment) is associated with bigger height gain, although a few studies refute that showing no correlation between height gain and age at puberty onset or initiation of treatment (20,59). Greater effectivenesss of GnRHa treatment on younger girls who are destined to poorer height prognosis without treatment, proves further that GnRH treatment is an effective strategy to preserve diminished height potential in these children.

BA advance at start of treatment and at the end, is negatively associated with height outcome (7,47,54,65,73). BA/statural age ratio at the onset of treatment and adult height is negatively associated with outcome suggesting that treatment is not capable of restoring a full adult height potential if started after a certain critical advancement of BA. Kauli et al (20) demonstrated that therapy is more beneficial if started before BA exceeds 12 years.

Height SDS at the onset (7,17,33,54,56,62,67) and at termination of treatment (7,17,54,56,59,67,73), as well as higher TH (7,17,65,67,72) have also been positively associated with adult height, supporting that influence of genetic factors on height is dominant among other factors.

Naturally, BA at the end of treatment, is associated with final height, as it determines posttreatment residual growth potential (7,59,71). Although data are scarce in this respect, stopping treatment at a BA of 12-12.5 years (7) or even <11.5 years (26) seemed to be associated with best height outcome, while continuing treatment after a BA ≥13 years negatively impacted on statural growth (7). Three factors explained 66% of adult height variance: BA advance before treatment, height at the end of treatment and height gain after interruption of treatment (33).

In summary, among the factors associated with the height outcome, height SDS and TH reflecting genetic potential, are always associated with positive outcome, BA advance and delay in treatment are negative factors. This highlights the importance of rapid recognition, evaluation and treatment of patients with true precocious puberty. However, one has to balance this with careful follow-up in some girls to not treat those with slow progression unnecessarily.

In terms of effcicacy of treatment, various GnRHa appeared similar as regards to height outcome (26,62,71,74), except for a study (69) demonstrating slightly better adult height SDS in patients treated with leuprolide depot compared to triptorelin depot.

Optimal Age of Discontinuation of Gonadotropin-Releasing Hormone Agonist Treatment in Girls with Central Precocious Puberty

Data is also missing on this respect. In the literature, (Table 6) the mean age at interruption of treatment ranges 9.4 to 12.7 averaging around age 11 year and BA ranging from 11.9 to 13.6 averaging 12.5 years. BA at the end of treatment correlates negatively with height gain after treatment. Carel et al (33) using multivariate analysis estimated that an 11 year old girl, growing 4 cm and gaining 0.5 BA year per year, could loose 2.6 cm of adult height if treatment was discontinued 1 year later. Opposite results were found by Klein et al (62) who found a positive correlation between age at discontinuation of treatment and adult height ($r = 0.25$, $p = 0.03$), suggesting that prolonging the treatment could increase height. Obviously, this discrepancy only can be solved with a formal controlled trial (i.e. randomizing girls between "early" and "late" age at discontinuation of treatment). However, such a trial would be difficult to perform since patients and the parents prefer to stop

Table 6. Height outcome in studies with gonadotrophin-releasing hormone agonists treatment of progressive idiopathic central precocious puberty

Author (ref no), year	n	CA at onset	BA at onset	CA at ET	BA at ET	TH	PH	FH	FH-TH	FH-PH
Kletter and Keltch (15), 1994	131	7.6 ± 0.13	10.9 ± 0.1			161.8 ± 0.7	155.9	157.9 ± 0.6	-3.9	2.0
		< 6 years 4.7 ± 0.3	9.4 ± 0.6			164.5 ± 1.4		160.4 ± 1.8	-4.1	
		> 6 years 8.1 ± 0.1	11.2 ± 0.1			161.4 ± 0.6		157.5 ± 0.6	-3.9	
Oostdijk et al (60), 1996	31	7.7	10.8	11.1	12.5	168.7 ± 6.4	158.2 ± 7.4	161.6 ± 7.0	-7.1	3.4
Kauli et al (20), 1997 (AD-AV)	48	8.3 ± 1.5	12.5 ± 0.7	11.5 ± 0.5	12.5 ± 0.7	157.7 ± 5.7	AD: 156.6 ± 6.7 AV: 152.3 ± 6.0	159.6 ± 6.3	1.9	3.0 7.3
	28	Unt 7.8 ± 1.0	Unt 10.2 ± 1.3			Unt 159.3 ± 6.1		Unt 155.5 ± 7.5	-3.8	
Bertelloni et al (26), 1998	14	6.2 ± 1.8	9.6 ± 1.6			163.3 ± 6.2	153.5 ± 7.2	158.1 ± 5.2	-5.2	4.6
Galluzzi et al (61), 1998	22	7.3 ± 1.1	10.3 ± 0.9	11.3 ± 0.7	12.6 ± 0.8		155.2 ± 4.7	158.5 ± 5.3		3.2
Carel et al (59), 1999	58	7.5 ± 1.3	10.1 ± 1.5	11.2 ± 1.0	12.2 ± 0.8	160.1 ± 4.4	156.4 ± 6.3	161.1 ± 5.9	1.0	4.7
Heger et al (64), 1999	50	6.2 ± 2.0	9.3 ± 2.5	11.0 ± 1.1		163.6 ± 6.2	154.9 ± 9.6	160.6 ± 8.0	-2.0	5.7
Arrigo et al (7), 1999	71	7.0 ± 1.3	9.8 ± 1.4	11.0 ± 1.0	12.4 ± 0.8	161.5 ± 6.9	155.5 ± 7.0	158.4 ± 5.8	-2.9	2.9
Léger et al (22), 2000	9	8.7 ± 0.4	11.1 ± 0.4	10.8 ± 0.6	11.8 ± 0.5	159.8 ± 4.6	155.3 ± 5.6	160.2 ± 6.7	0.4	4.9
Partsch et al (16), 2000	52	6.2 ± 0.3	9.3 ± 0.3	11.1 ± 1.1	12.6 ± 0.2	164	154.9 ± 9.6	160.6 ± 8.0	-3.4	5.7
		< 6 years 5.0 ± 0.35				162.4 ± 1.08 165.3 ± 1.43	152.1 ± 2.22 157.7 ± 1.80	161.6 ± 1.43 159.4 ± 1.75	-0.8 -5.9	9.6 1.7
		> 6 years 7.8 + 0.18								
Klein et al (62), 2001	80	5.4 ± 1.9	10.0 ± 2.7	11.1 ± 1.0	12.8 ± 1.1	163.7 ± 5.6	149.3 ± 9.6	159.8 ± 7.6	-3.9	10.5
	< 6 yr					164.5 ± 5.9	NA	162.1 ± 7.0	-2.4	14.5
	> 6 yr					NA	151.1 ± 8.6	157.9 ± 7.6	NA	6.8
Bajpai et al (65), 2002 (AV)	30	6.5 ± 1.8	10.1 ± 1.6	10.2 ± 2.5	12.0 ± 0.5	154.7 ± 6.1	143.4 ± 8.3	149.8 ± 6.9	-4.9	6.4

Table 6. Continue

Antoniazzi et al (23), 2000	15	7.6 ± 0.5	9.8 ± 1.0	11.0 ± 0.9	12.1 ± 0.8	157.6 ± 5.9		160.6 ± 5.7	3.0	
	10	Unt 7.2 ± 0.9	Unt 9.6 ± 2.2			Unt 156.4 ± 1.3		Unt 149.6 ± 6.3		
Adan et al (47), 2002	43	7.9 ± 1.3	10.3 ± 1.3	10.8 ± 0.7	12.2 ± 0.7	161.2 ± 4.6	156.0 ± 7.8	159.5 ± 5.3	-1.7	3.5
Tanaka et al (54), 2005 (AD-AV)	63	7.7 ± 2.2	10.2 ± 1.5	11.6 ± 1.4	12.0 ± 0.8	154.9 ± 4.6	AD: 154.5 ± 7.1	154.5 ± 5.7	-0.4	0
							AV: 151.1 ± 7.3			3.4
Tung et al (66), 2007	11	8.0 ± 1.5	11.5 ± 1.3	12.7 ± 0.9	13.6 ± 0.6	157.0 ± 4.5	146.7 ± 4.8	156.3 ± 4.3	-0.7	9.6
Lazar et al (17), 2007	60	< 6 years 6.4 ± 1.2	8.9	11.3 ± 0.4	12.1 ± 0.5	159.3 ± 5.0	154.6 ± 6.6	162.8 ± 5.0	3.1	8.2
		> 6 years 7.5 ± 0.6	10	11.3 ± 0.3	12.4 ± 0.5	157.8 ± 5.2	153.7 ± 6.7	157.9 ± 5.1	4.2	0.1
Pasquino et al (55), 2008 (AD-AV)	87	8.4 ± 1.5	11.1 ± 1.6	12.6 ± 1.0	13.1 ± 0.5	157.6 ± 4.7	AD: 154.2 ± 5.2	159.8 ± 5.3	2.2	5.6
							AV: 150.0 ± 5.1			9.8
Brito et al (67), 2008 (AD-AV)	45	7.3 ± 2.0	10.6 ± 2.2	10.7 ± 0.8	12.4 ± 0.9	157.5 ± 4.5	AD: 151.6 ± 9.7	155.3 ± 6.9	-2.2	3.7
							AV: 147.3 ± 9.0			8.0
Nabhan et al (68), 2009 (P)	26	7.2 ± 2.0	10.1 ± 2.2	10.9 ± 1.2	12.4 ± 0.9	164.0 ± 5.7	158.5 ± 6.8	163.0 ± 7.6	-1.0	4.5
Massart et al (69), 2009	47	L group: 7.6 ± 0.7	L group: 10.5 ± 1.3	Lgroup: 10.6 ± 1.1	L group: 12.3 ± 0.9	Ht SDS L group: -0.2 ± 0.3	Ht SDS L group: -1.0 ± 0.6	Ht SDS L group: -1.5 ± 0.7		
		T group: 7.4 ± 0.8	T group: 10.2 ± 1.3	T group: 9.4 ± 1.2	T group: 12.0 ± 0.7	T group: 0.1 ± 0.2	T group: -0.6 ± 0.6	T group: -0.9 ± 0.5		
Magiakou et al (28), 2010 (AD-AV)	33	7.92	10.0			158.75	AD: 158.16	158.5	-0.25	0.4
							AV:151.53			
	14	Unt 7.95	Unt 10.75			Unt 161.2 cm	Unt AD: 160.2	Unt 161.5		7.0
							AV: 154.3			
Lee et al (70), 2011	29	7.3 ± 1.9	10.2 ± 2.13			163.8	157.4	162.5	-1.3	5.1
Poomthavorn et al (56), 2011 (AD-AV)	47	8.5 ± 1.0	11.1 ± 1.7	11.8 ± 1.0	13.3 ± 0.5	155.8 ± 4.1	AD: 155.3 ± 6.7	158.6 ± 5.2	2.8	3.3
							AV: 150.8 ± 5.5			7.8

Table 6. Continue

Gillis et al (71), 2013 (AV)	23	T group: 8.4 ± 0.3	10.0 ± 0.3	10.6		160.8 ± 0.8	155.2 ± 1.9	157.9 ± 1.7	-0.9	2.7
		HIS impt group: 8.7 ± 0.3	10.4 ± 0.4	11.7		160.1 ± 1.0	156.8 ± 2.6	161 ± 2.0	1.0	4.3
	11									
Jung et al (72), 2014	59	8.7 ± 0.8	10.2 ± 1.6	10.6 ± 0.8	11.9 ± 0.5	159.9 ± 3.5	156.6 ± 4.0	160.4 ± 4.2	0.5	3.8
Atay et al (75), 2014	48	7.76 ± 1.2	9.66 ± 1.4			159.0	154.6	160.6	1.6	6.0
	52	Unt: 8.08 ± 0.8	Unt: 10.0 ± 1.5			Unt: 160.0		Unt: 158.1	Unt: -1.9	
Liang et al (73), 2015	17	8.1 ± 0.2	9.2 ± 0.3			158.3 ± 0.9	161.6 ± 0.9	159.8 ± 1.2	1.5	-1.8
Bertelloni et al (74), 2015 (AD-AV)	13	Three monthly: 7.9 ± 0.6	10.6 ± 0.9			159.7 ± 3.8	AD: 155.0 ± 3.5 AV: 149.9 ± 3.5	157.1 ± 4.9	-2.6	2.1 7.2
						158.4 ± 5.0			-0.3	
	12	Monthly: 8.0 ± 0.6					AD: 155.4 ± 5.9 AV: 150.2 ± 5.3	158.1 ± 6.6		2.7 7.9
			10.4 ± 0.9							

n: number of subjects, CA: chronological age, BA: bone age, ET: end of treatment, TH: midparental target height, PH: predicted height at treatment initiation, FH: final height, T: triptorelin depot, Unt: untreated control group, NA: not available, L: leuprolide, HIS impt: histrelin implant, Ht SDS: height standard deviation score, AV: PH calculated according to average tables, AD: PH calculated according to tables for advanced bone age; if not specified, advanced tables were used, P: PH calculated according to the model proposed by "Post E, Richman R. A condensed table for predicting adult stature. J Pediatr 1981;98:440-442"

All values are presented as mean ± standard deviation when available. Height is expressed in centimeters, age in years, IN: intranasal

treatment when the girl has reached an age that peers of the patients have already started puberty which is usually around age 11 year.

Could the BA be a useful parameter to decide when to stop treatment? Although the optimal age for treatment interruption is not clearly defined by international guidelines, it has been proposed that the best heights are achieved when treatment is discontinued at around 12-12.5 years in girls (7,76) However, in girls around the age of 11 years with previous advance in BA and a long-standing treatment with GnRH agonists, BA often is approximately 12 years with little variation and is therefore of little help to orient decisions. Furthermore, reduction of growth velocity, commonly observed around this age, due to the increasing dependence of growth on sex steroids (77) with time, necessitates stopping treatment.

Treatment of Gonadotropin-Releasing Hormone Analogues Combined with Growth Hormone Treatment (Table 7)

The growth velocity in some CPP patients decreases below the normal for prepubertal children during GnRHa therapy. Subnormal growth velocity during GnRHa therapy may be associated with a decrease in GH and insulin-like growth factor 1 secretion due to suppression of gonadal steroids (77). Therefore, some studies investigated in girls with precocious puberty and poor predicted height, whether adding GH to GnRHa treatment is associated with a better height outcome (78,79,80,81,82,83,84). Data is even more limited and biased about this type of approach. In short, it can be stated that at present, studies are insufficient to make definite conclusions about the height outcomes of GnRHa plus GH treatment. Lanes and Gunczler (78) treated 15 short children (boys and girls) entering into normally timed puberty with both GnRHa and GH and compared them with an identical number of untreated children. In their study, no relevant height gain was observed after 2.5 years of treatment.

Pasquino et al (79) and Pucarelli et al (80) on the other hand, showed differences of about 6-8 cm of height gain on girls with CPP treated with GnRHa plus GH, compared to

Table 7. Studies investigating gonadotrophin-releasing hormone analog plus growth hormone treatment on final height of girls with central precocious puberty

Author (ref), year	Comparison	Treatment	Duration of therapy (year)	Subjects (n)	CA at onset	BA at onset	TH	PAH	FH	FH-PAH	FH-TH
Pasquino et al (79), 1999	GnRHa	Triptorelin, 100 mg/kg/q 21 days	4.9	10	7.6 ± 0.2	10.4 ± 0.3	155.5 ± 2.1	155.5 ± 2.0	157.1 ± 2.5	1.6 ± 1.2	1.6
	GnRHa + GH	Triptorelin + GH 0.3 mg/kg/week (after 2-3 years of GnRH therapy)	5.1 (total)	10	7.9 ± 0.6 10.0 ± 0.5#	10.6 ± 0.4& 12.0 ± 0.2#	155.6 ± 2.0	152.7 ± 1.7	160.6 ± 1.3	7.9 ± 1.1	5.0
Pucarelli et al (80), 2003	GnRHa	Trip 100 mg/kg/21 days, i.m.	2-4 years	18	7.9 ± 0.8	10.7 ± 1.2	157.2 ± 6.0	153.9 ± 3.8 AD 149.6 ± 4.0 AV	156.6 ± 5.7	2.3 ± 2.9 AD	-0.6 3.8
	GnRHa + GH	Triptorelin + GH 0.3 mg/kg/week		17	9.9 ± 1.3	12.1 ± 0.8	157.4 ± 4.8	156.2 ± 4.5 AD 150 ± 4.5 AV	161.2 ± 4.8	8.2 ± 4.8 AV	
Tuvemo et al (82), 2004	GnRHa	Buserelin 300 mg/6 times daily, IN 2-4 years	2-4 years	22	8.2 ± 0.83	NA		NA	155.8 ± 6.9		
	GnRHa + GH	Buserelin + GH 0.033 mg/kg/week		24	8.4 ± 0.78	NA			158.9 ± 5.4		
Mul et al (81), 2005	GnRHa	Triptorelin 3.75 mg/28 days, i.m.	3 years	12	9.6 ± 0.9	10.7 ± 1.1	NA	156.0 ± 5.7 AD 149.8 ± 5.6 AV	155.0 ± 5.6	-1.0 ± 3.6 5.2 ± 3.7	
	GnRHa + GH	Triptorelin + GH 4 IU/m^2/day, i.m.		14	9.6 ± 0.9	11.6 ± 0.8		151.7 ± 5.0 AD 146.8 ± 4.8 AV	155.0 ± 5.5	3.3 ± 3.5 8.2 ± 3.4	
Jung et al (83), 2014	GnRH	GnRHa* 75-150 µg/kg q 28 days	2 years	59	8.7 ± 0.8	10.2 ± 1.6	159.9 ± 3.5	156.6 ± 4.0	160.4 ± 4.2	3.8	0.5 1.2
	GnRHa + GH	GnRHA + GH 0.25 mg/kg per week		23	8.8 ± 0.59	10.5 ± 0.86	158.1 ± 3.31	154.6 ± 2.55	159.3 ± 5.33	4.7	

*Gonadotrophin-releasing hormone analog not specified, and at the beginning of gonadotrophin-releasing hormone agonists therapy, #at the beginning of growth hormone therapy

AD: predicted height at treatment initiation calculated according to tables for advanced bone age, AV: predicted height at treatment initiation calculated according to average tables, GnRH: gonadotrophin-releasing hormone, GnRHa: gonadotrophin-releasing hormone agonists, GH: growth hormone, CA: chronological age, BA: bone age, TH: midparental target height, FH: final height, PAH: predicted adult height, i.m.: intramuscular, NA: not available, IN: intranasal

GnRHa alone. In their first report, the gain in centimeters, (calculated between pretreatment PAH (152.7 ± 1.7 cm) and final height (160.6 ± 1.3 cm), was 7.9 ± 1.1 in patients treated with GH plus GnRHa, whereas in patients treated with GnRHa alone, the gain between pretreatment PAH (155.5 ± 1.7) and final height (157.1 ± 2.5 cm) was just 1.6 cm ± 1.2. The difference between the gain obtained in the groups is significant, in favor of combination group ($p < 0.001$) (79). However, the same group reported four years later a larger number of patients with a longer follow-up period that, adult height versus pre-treatment PAH was 6 cm greater in combination treatment than that of GnRHa alone but concluded that true efficacy of the addition of GH to GnRHa therapy is still questionable (80). They recommended caution regarding such an invasive and expensive treatment, outside a research setting.

It should also be taken into account that, in the above studies, the treatment period was not standardized, and the authors treated a selected group of patients, i.e. those whose height velocity decreased to value < p25 for chronological age under GnRHa treatment. Besides, the duration of treatment in these studies was remarkably longer than in other studies with combined treatment and GH dosage was higher.

A randomized controlled study, in short adopted girls with early puberty, Mul et al (81) treated girls with onset of puberty before 10 years of age for 3 years with either GnRHa alone (group A, n = 12) or with GnRHa and GH (group B, n = 14). Height gain defined as the difference between initial height prediction and attained final height, was significantly different between group A and B (5.2 ± 3.7 cm and 8.2 ± 3.4 cm, $p < 0.05$) using average tables for height prediction. However, with advanced tables for height prediction, the numbers were much less (-1.0 ± 3.6 and 3.3 ± 3.5 cm, respectively).

A recent Korean study in 82 girls with idiopathic CPP showed a height gain of approximately 3.8 cm in the GnRHa alone group, while 4.7 cm in the combination group compared to PAH before treatment with no statistically significant difference between two groups. (83). Finally a recent meta-analyses, evaluating a total of six randomized controlled trials (RCTs) (162 patients) and six clinical controlled trials (CCTs) (247 patients) reported that compared to the GnRHa therapy group, the combination therapy group achieved taller final height (mean difference = 2.81 cm, four CCTs and 4.30 cm, in one RCT); and 3.9 cm better final height compared with THs (84).

The results of these studies (comparing adult height vs. predicted height) should again be interpreted in the context of the before mentioned methodological handicaps of accurately predicting adult height. Furthermore, the number of treated patients are much less, and most likely involves selection biase as those who have poor height potential or attenuated growth velocity might tend to choose or given the combined GnRHa GH treatment. Finally, since GH treatment requires the consideration of cost, economic status may be another affecting factor to select the patients treated with GnRHa plus GH. Cost-effectiveness of combined GH treatment in patients with CPP has also to be elucidated.

Ethics

Peer-review: Internally peer-reviewed.

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REVIEW

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Insulin Resistance, Prediabetes, Metabolic Syndrome: What Should Every Pediatrician Know?

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Abstract

The Metabolic syndrome describes a clustering of typical cardiovascular risk factors. The syndrome is also known as "Insulin Resistance syndrome" as a substantial part of the pathophysiology is driven by resistance to the metabolic effects of insulin. The major cause of insulin resistance in childhood is a typical lipid partitioning pattern characterized by increased deposition of lipids within insulin responsive tissues, such as the liver and skeletal muscle and within the viscera. This lipid deposition pattern is also associated with infiltration of intra-abdominal tissues with cells of the immune system, inducing systemic, low-grade inflammation typically observed in insulin resistant obese children and adolescents. Several clues derived from a careful history and physical examination, along with a basic laboratory workup, provide clues in regards to risk stratification in obese children.

Keywords: Obesity, children, Metabolic syndrome, prediabetes, insulin resistance

Introduction

The Metabolic syndrome, also known as Insulin Resistance syndrome or syndrome-X, describes cardiovascular risk factor clustering (CVRFC) in specific individuals (1). The reason for describing these as a syndrome rather than individual and independent risk factors is that they are postulated to be driven by a shared pathophysiological mechanism. The clinical significance of this syndrome is very well established in adults, confirming a significantly increased risk for the development of type 2 diabetes mellitus (T2DM) and coronary heart disease over time (2). While the adult definition of the Metabolic syndrome is well established and can easily be used for clinical purposes, the definition in the pediatric age group is controversial, less stable over time and is harder to utilize clinically (3). There are several reasons for this difficulty, stemming from the normal changes in body proportions in growing children, hormonal effects of normal pubertal development on some of the criteria defining the syndrome and a different balance of the factors governing glucose metabolism between obese children and adolescents compared to adults (4). Some argue that for clinical purposes, the definition of the syndrome should not be utilized and its individual components should be addressed separately (5). This may be true for conveying a clear message to the child and parents, yet the caregiver must understand that there is a common shared mechanism driving the pathophysiology of the development of separate components of the syndrome and that this mechanism should be addressed in order to provide a beneficial clinical outcome. In this review, we first describe the pathophysiology of the syndrome and later provide key clinical insights relevant to the pediatrician.

Pathophysiology of Insulin Resistance

Reaven (6) was the first to provide a physiological mechanism for the clustering of obesity, dyslipidemia, hypertension and altered glucose metabolism. Reaven (7) suggested that insulin resistance (IR), manifesting as hyperinsulinemia, is the driving factor for the development of dyslipidemia, elevated blood pressure and altered glucose metabolism. As obesity is commonly associated with IR (and is the main cause of IR in childhood), this anthropometric parameter, described using either body mass index (BMI) or waist circumference, serves as part of the syndrome definition. Importantly, there is no uniform definition of insulin sensitivity/resistance. The reason for this is that there is still no standardized assay for measurement of plasma insulin

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(that must be used to define insulin sensitivity) thus it is difficult to compare results between laboratories using different assays. Moreover, the "gold standard" methodology for measurement of whole body insulin sensitivity is the euglycemic-hyperinsulinemic clamp (8). In this method, a standardized (per body surface area or body weight) insulin infusion is delivered to a fasting patient while in parallel-glucose is infused in order to maintain glucose concentration at a "clamped" fasting level. The steady state glucose infusion rate (in some cases adjusted for ambient insulin concentrations) achieved at the last 30 minutes of the study is defined as the insulin sensitivity of the patient. However, this methodology is used for research purposes only and is not practical for clinical use. Several surrogate indices of whole body insulin sensitivity/resistance have been developed using oral glucose tolerance tests, such as the Matsuda index (9) and fasting samples [such as the homeostatic model for assessment of IR, Homeostatic Model of Assessment-IR (HOMA-IR)] (10). These surrogates have been shown to moderately correlate with "gold standard" measurements in obese, but not necessarily in non-obese, children and adolescents (11), thus their clinical utility is at present not proven. The definition of IR in physiological terms is that greater concentrations of insulin are needed to elicit a physiological effect that was previously induced by lower concentrations of the hormone. Of note, the main factor determining insulin concentrations is its effect on glucose metabolism. Thus, greater plasma glucose, whether derived from endogenous (hepatic glucose production) or exogenous (dietary) sources will result in higher insulin concentrations assuming that beta cell capacity is preserved, which is not the case in patients with diabetes. Insulin sensitivity differs between several insulin-responsive organs so that, for example, in certain conditions hepatic glucose production may be adequately suppressed while muscle glucose uptake may be low on exposure to the same insulin concentration. Moreover, IR in the context of Metabolic syndrome may be present specifically in the insulin signal transduction pathway related to glucose metabolism within a tissue but not in other intracellular elements of this pathway related to other functions such as lipid metabolism or proliferation. For example, this may mean that the resistance to insulin in the suppression of the liver gluconeogenesis pathway could result in higher systemic insulin concentrations yet the response of parallel effects of insulin within the liver [such as very low density lipoprotein (VLDL) synthesis] may not be impaired and thus respond adequately to the higher insulin concentrations by increasing the metabolic flux within that segment of the pathway (12). The main insulin-responsive tissues related to glucose metabolism are the liver, skeletal muscle and adipose tissue. Under fasting conditions, hepatic glucose production is regulated by basal insulin levels while muscle uptake of glucose from the plasma is low and adipose tissue provides free fatty acids (FFAs) via lipolysis as an energy source. In post-prandial conditions, that is when insulin levels are elevated, hepatic glucose production and adipose lipolysis are suppressed while muscle glucose uptake is increased. This is achieved by suppression of gluconeogenesis and glycogen breakdown in the liver and by increased trafficking of the glucose transporter type 4 in muscle. In post prandial conditions, lipogenesis is activated in adipose tissue and lipolysis is suppressed. As indicated earlier, the main regulator of insulin secretion is plasma glucose concentration. If, for example, there is increased IR in skeletal muscle, greater insulin concentrations will be necessary to induce muscle glucose uptake. If hepatic IR is present (i.e., resistance in the insulin signal transduction pathway regulating gluconeogenesis), greater basal insulin concentrations will be necessary to maintain normal fasting glucose levels. Both examples, which usually occur concurrently to some degree, result in relative hyperinsulinemia to which all tissues and organs will be exposed. In this scenario, metabolic pathways regulated by insulin but not necessarily related to glucose will be activated in excess, as there is no resistance in those elements of the insulin signal transduction pathway. For example, in the kidney, insulin stimulates increased sodium reabsorption. In the face of systemic hyperinsulinemia, this will result in excess sodium reabsorption, leading to increased intravascular volume and potentially to elevated blood pressure. It has been shown that insulin resistant individuals have an impaired natriuretic response to increased sodium intake (13), typical of a diet rich in processed food. Similarly, exposure of specific brain nuclei to hyperinsulinemia results in an increased sympathetic discharge, manifesting similarly in elevated blood pressure (14). In the ovaries, theca cells have insulin receptors that respond minimally to normal basal insulin concentrations. However, under conditions of hyperinsulinemia, these receptors induce androgen production resulting in hyperandrogenism (clinically manifesting in hirsutism, oligomennorhea and polycystic ovaries) (15). In the liver, while elevated insulin concentrations may be needed to regulate hepatic glucose production, hepatic, insulin-responsive lipogenesis mechanisms have no resistance and are hyper-activated, resulting in increased VLDL and reduced high density lipoprotein (HDL) particle production, manifesting as increased plasma triglycerides and low HDL-cholesterol concentrations (16,17). Thus, multiple manifestations of the IR syndrome are the result of a normal response of metabolic pathways to increased insulin concentrations that are

induced in order to maintain normal glucose metabolism. The reasons for development of IR in insulin responsive tissues are multiple and complex. The common paradigm of this process suggests that accumulation of intracellular lipid (probably via long chain fatty acyl-coenzyme A) induces inhibition of specific components of the insulin signal transduction pathways related to glucose metabolism in liver and muscle (18,19). It is well established that increased intra-myocellular and intra-hepatic lipid are tightly associated with peripheral and hepatic IR respectively (20). Moreover, it has been shown that infusion of intravenous FFAs during a hyperinsulinemic-euglycemic clamp results in an acute reduction of insulin sensitivity (21). Additional factors that may cause acute reductions in liver and muscle insulin sensitivity are an inflammatory stress response, such as that induced by an acute infection or by the use of systemic steroids (22,23,24). In subjects with diabetes, exposure to such stress will result in acute hyperglycemia while in children with normal glucose metabolism these types of stimuli can lead to transient hyperinsulinemia, needed to maintain euglycemia, accompanied by elevated triglycerides. An additional factor linking obesity to increased IR is systemic inflammation (25,26). It is well established that subcutaneous and intra-abdominal lipid depots may be infiltrated by cells of the immune system (mainly macrophages) that have the potential to induce local, as well as systemic, inflammatory activation. Inflammation of hypothalamic nuclei in this scenario may further exacerbate metabolic derangement (27). Similar to fatty acid derivatives within muscle and liver cells, inflammatory cytokines can adversely affect the insulin signal transduction pathway leading to IR. Chronic stress, such as that of chronic disease or emotional stress may have similar effects on systemic insulin responses, resulting in a reduction in whole body insulin sensitivity manifesting as hyperinsulinemia (28). Importantly, the normal physiological hormonal changes of puberty lead to a transient yet substantial reduction in whole body insulin sensitivity during mid-puberty which may resolve by the end of puberty (29,30). Moreover, the impact of sex hormones on components of the Metabolic syndrome may differ between males and females (31). This has relevant implications for the assessment of components of the Metabolic syndrome e, as some may be transiently abnormal in mid-puberty and normalize by the end of puberty. This phenomenon is well established and its significance and impact on the stability of the relevant measurements is a matter of debate (32). Thus, whole body IR manifests clinically in different organs depending on the degree of response to insulin of signal transduction pathways that are not necessarily involved in glucose metabolism. For example, this may manifest as increased activity of the lipoprotein synthetic pathway in the liver (which responds normally to higher systemic insulin concentrations) or in a greater sympathetic discharge. In addition, the insulin resistant, obese child typically shows biochemical evidence of subclinical systemic inflammation.

Implications of the Pathophysiology of Insulin Resistance on the Clinical Approach to the Obese Child

It is important to identify obese children and adolescents suspected of having an underlying organic cause for their obesity and those who have any obesity related major comorbidities. This group of patients should be referred to a pediatric obesity specialist. In the vast majority of children, the cause of overweight or obesity is a combination of genetic predisposition and environmental factors such as sedentary lifestyle and increased consumption of calorie rich food (33). The proportion of obese children with an organic cause for their weight gain is very low, even within specialist obesity clinics, yet these should be identified and managed appropriately. The primary care physician is responsible for identifying children who are overweight and assessing their vulnerability for developing obesity and its complications (33). This translates into a risk stratification strategy that is aimed at identifying those at greatest risk for present and future obesity related morbidity and focusing on their management.

Personal Medical History (Table 1)

It is evident that personal medical history details that may raise suspicion of an organic cause of obesity should be sought. Recent accelerated weight gain, substantial weight gain starting in infancy (specifically if accompanied by dysmorphic features), easy bruising, exposure to exogenous corticosteroids, headaches, changes in vision or other clues that raise suspicion of an intracranial lesion, of Cushing's syndrome or of hypothyroidism should be sought. These are very uncommon causes of obesity and IR, yet should not be missed (33). Starting with pregnancy history, focused questions about maternal gestational diabetes mellitus (GDM), hypertension or any intrauterine growth retardation are crucial for the assessment of the obese child. It is well

Table 1. Clues in the history for metabolic syndrome

Maternal gestational diabetes	Types of food, sweetened beverages?
IUGR	Eating habits
Birth weight, SGA	Sleep patterns, snoring
Catch-up growth	Polyuria, polydipsia
Family history of T2DM, CVD	Medications

IUGR: intrauterine growth retardation, T2DM: type 2 diabetes mellitus, SGA: small for gestational age, CVD: cardiovascular disease

described that being born small for gestational age (SGA) is associated with a specific pattern of post-natal changes in body composition reminiscent of the lipid partitioning pattern described previously (34). Specifically, those born SGA tend to develop greater intra-abdominal lipid deposition than their appropriate for gestational age counterparts, even prior to the development of obesity, and are at high risk for the presence of the syndrome in adolescence (35,36,37). Moreover, having a head circumference smaller than the 10th centile at birth, indicating significant intrauterine growth retardation, is associated with an increased risk of developing manifestations of IR, and specifically T2DM, in early adulthood (35). Exposure to GDM *in utero* has been shown to affect beta cell function of the developing fetus (38). Obese children who have been exposed to GDM show poorer insulin secretion compared to equally obese children who were not exposed. Being exposed to the hyperglycemic milieu of GDM along with the obligate genetic components associated with T2DM inherited from the parents confer an increased risk of IR in general, and beta cell dysfunction in particular, in the developing fetus which will manifest later in life (39). It is crucial to assess previous anthropometric data using appropriate growth charts and pay attention to the catch-up growth of smaller babies. Accelerated catch-up growth within the first year of life in general and specifically within the first months has been shown to be associated with the lipid partitioning pattern described above which confers greater metabolic risk (40). This is particularly relevant for infants who were born SGA. Infants and older children presenting with significant obesity that developed before the age of five years, and specifically those with significant weight gain in the first year of life, are more suspicious of genetic causes for their obesity. Upon comparing adolescents who were equally obese yet differed in their metabolic phenotype (those with vs. those without the presence of the Metabolic syndrome), it has been shown that early development of obesity as well as length of exposure to obesity were both associated with an adverse metabolic profile (41). While overall caloric intake is very important, specific elements of the diet have been shown to have a greater impact on weight gain and the metabolic profile of the obese child. Specifically, consumption of sweetened beverages has been shown by most studies to be associated with greater risk of obesity in childhood (42). Reduction, and ideally elimination, of sweetened beverages from the diet of an obese child is the basic first step in dietary modification (43). It has been shown that across the globe children may consume a substantial amount of their daily caloric input from such beverages and changing this habit may have a substantial impact on weight and metabolic risk. Fructose consumption has been claimed to be the culprit of lipid deposition in the liver, resulting in increased whole body IR. Short-term substitution of fructose (by eliminating simple sugars in the form of sucrose) with starch, without a change in total calories per day, has recently been shown to reduce intra-hepatic fat and result in significant improvements in glucose and lipid metabolism (44). Thus, specific attention should be paid, when taking the dietary history, to identification of the components of the diet whose change may lead to metabolic improvement even without significant weight reduction. Specific questioning should be devoted to the sleep pattern of the obese child. Firstly, the length of sleep and the ease of awakening should be assessed. Obesity is associated in some cases with an increased tendency to develop obstructive sleep apnea (OSA). OSA has been shown to be associated with further development of IR, probably due to sympathetic activation (45). Thus, children that snore or are suspected of having sleep apnea should undergo a polysomnographic assessment with optional intervention where appropriate. Sleep time should also be assessed as reduced sleep time has been shown to be associated with tendency for obesity (33). Assessment of the physical activity and sedentary behavior of the obese child is mandatory. Greater than 2 hours per day of "media time" (including television, computer and cellphone exposure) have been shown to be associated with a greater risk of obesity. Conversely, any form of physical activity, which does not need to be intense, may have a beneficial impact on body composition and whole body insulin sensitivity. Thus, physical activity may not necessarily lead to weight loss but will have beneficial metabolic effects and should thus be encouraged (46). A thorough history of medication use is also mandatory in obese children. Some medications such as systemic steroids may have a significant adverse impact on weight and on insulin sensitivity (23). Psychotrophic medications, specifically novel anti-psychotics, have been associated with significant weight gain and IR, which is usually observed soon after their initiation (47). A careful family history of children at risk for the presence of Metabolic syndrome is crucial. The syndrome has a strong genetic component as evidenced by twin, as well as parental, studies (48). It is well established that young, lean offspring of parents with type 2 diabetes have greater intramuscular fat and lower whole body insulin sensitivity compared to their counterparts without such family history (49).

Physical Examination (Table 2)

Accurate anthropometric measurements should be performed in any child and this is equally important in obese children. In addition to plotting standard height, weight and BMI, waist circumference should be measured and, if

possible, plotted on appropriate charts. A standard, complete physical examination is mandatory. Specific attention should be given to the presence of dysmorphic features as these, along with weight gain and obesity development in infancy and early childhood, raise more suspicion of genetic obesity syndromes. The presence of acanthosis nigricans (AN) should be sought. The presence of AN, usually seen on the neck and in skin folds, signifies exposure of acanthocytes to hyperinsulinemia (probably interacting with insulin-like growth factor-1 receptors on these cells) (50). Blood pressure should be measured using an appropriately sized cuff. Reference values should be those adjusted for age, sex and height. As some patients suffer from "white coat hypertension", measurements should be performed in a relaxed and quite atmosphere. If blood pressure levels are elevated on repeated measurements, a 24-hour ambulatory evaluation using a blood pressure halter should be considered. As in any pediatric examination, Tanner staging of pubertal development should be performed, as mid-puberty is characterized by reduced whole body insulin sensitivity. Table 2 shows the main elements to be examined by systems. In addition to these, anthropometric measurements should be recorded (weight, height, waist circumference and calculating BMI).

Laboratory Workup (Table 3)

The laboratory workup of an obese child suspected of having biochemical manifestations of IR should aim to identify the presence of subclinical clues to this state. A biochemistry panel that includes fasting lipids as well as liver function studies should always be requested. Elevated triglycerides and reduced concentrations of HDL-cholesterol are typical manifestations of IR and are components by definition of the Metabolic syndrome. Moreover, the ratio of triglycerides to HDL-cholesterol is a simple biomarker that identifies increased IR in this age group (51,52). When measured in mg/dL, a ratio greater than 2.25 is a marker of increased IR. Elevated alanine amino transferase (ALT) concentrations, typically less than double the normal range, and without an accompanying elevated aspartate aminotransferase level should raise suspicion of hepatic steatosis (53). However, normal ALT concentrations do not rule out increased hepatic lipid deposition. A urinary sample for the presence of microalbuminuria should be evaluated as early defects in vascular function typically accompany obesity in childhood and are also associated with an adverse metabolic phenotype (54). Thyroid function and an early morning cortisol should be performed as screening for hypothyroidism and Cushing's, respectively. Other biochemical or hormonal studies should be sent based solely on individual clinical suspicion and should not be performed routinely in all obese patients. The evaluation of glucose metabolism in the obese child is more complicated. Most adult and pediatric definitions of the Metabolic syndrome include a fasting glucose. Fasting glucose is easy to measure, yet impaired fasting glucose (IFG) is only one of the manifestations of altered glucose metabolism in childhood and probably not the major one. Elevated two hour glucose, that is impaired glucose tolerance (IGT), is a stronger predictor of the development of T2DM and of the presence of atherogenic lesions in major arteries in children (55,56). IGT in obese youth is associated with a reduction of first phase insulin secretion, the earliest metabolic lesion that predicts future development of T2DM (57). The evaluation of two hour glucose involves performance of an oral glucose tolerance

Table 2. Main elements in the physical examination of the obese child evaluated for the presence of cardiovascular disease risk factors

General appearance	Obesity pattern, intra-abdominal type (apple shape) vs extremity type (pear shape), document waist circumference and calculate BMI, Perform Tanner staging
Skin	Hyper/hypopigmentation lesions, purple abdominal striae, acanthosis nigricans, signs of virilization in females.
Respiratory system	Ask about snoring and any sign of upper airway obstruction, dyspnea.
Cardiovascular	Assess blood pressure with appropriate size cuff, resting tachycardia
Abdomen	Measure waist circumference, look for striae, organomegaly

BMI: body mass index

Table 3. Laboratory tests for evaluating obese children

CBC	Urinary sample for microalbuminuria
Fasting glucose/oral glucose tolerance test, where appropriate	Thyroid function
Complete biochemistry (including ALT, GGTP)	Early morning cortisol
Fasting lipid profile	

ALT: alanine amino transferase, GGTP: gamma-glutamyl transpeptidase CBC: complete blood count

test. This test may uncover substantial defects in glucose metabolism that might be missed using only a fasting glucose. Importantly, a mid pubertal child may have prediabetes (indicated by either IFG or IGT), which may revert to normal on repeated testing after completion of puberty. This is due to the transient rise in IR during mid-puberty. Albeit being a common phenomenon, this indicates that when being faced with a certain degree of IR, the patient's beta cell fails to compensate appropriately. This means that while the patient has normal glucose metabolism upon repeated measurements, future decreases in insulin sensitivity (such as physiological ones during pregnancy and normal aging or conditions such as acute diseases, use of corticosteroids and others) may unravel such beta cell defects and manifest as altered glucose metabolism. In an obese child with multiple risk factors, such as a family history of T2DM, AN and/or high waist circumference, an oral glucose tolerance test should be performed as only a fasting glucose my be diagnostically inadequate. Measurement of a fasting insulin level is not recommended by most authorities (34). As the assays for insulin measurement are not standardized, it is difficult to evaluate measurements in comparison to any reference values. Calculation of a HOMA-IR in obese children does not add meaningful clinical information because the correlation between the HOMA-IR and "gold standard" measurements of insulin sensitivity is poor (11). Moreover, slightly elevated insulin concentrations may be present across the entire spectrum of whole body insulin sensitivity in obese children and thus provide no meaningful information that may affect treatment decisions. The only utilization of fasting insulin levels may be for longitudinal follow up of individual patients. Yet the additional information this will potentially add is probably modest and adds little beyond measurement of standard Metabolic syndrome components and standard biochemistry testing.

Novel biomarkers, such as adiponectin levels, may provide strong evidence for the presence of IR and altered lipid partitioning (58,59). These molecules are not yet used for standard clinical care yet provide valuable insights into the metabolic phenotype of the obese child. Figure 1 relates the pathophysiology associated with obesity to the parameters of the Metabolic syndrome and summarizes the relationships between them.

How Do We Interpret the Results of Our Workup?

After investigation of relevant anthropometric and biochemical markers, a risk stratification evaluation of the obese child can be performed. Using the most relevant Metabolic syndrome definition available and relevant to the patient, the presence of risk factors can be quantified. The most appropriate simple definition of the Metabolic

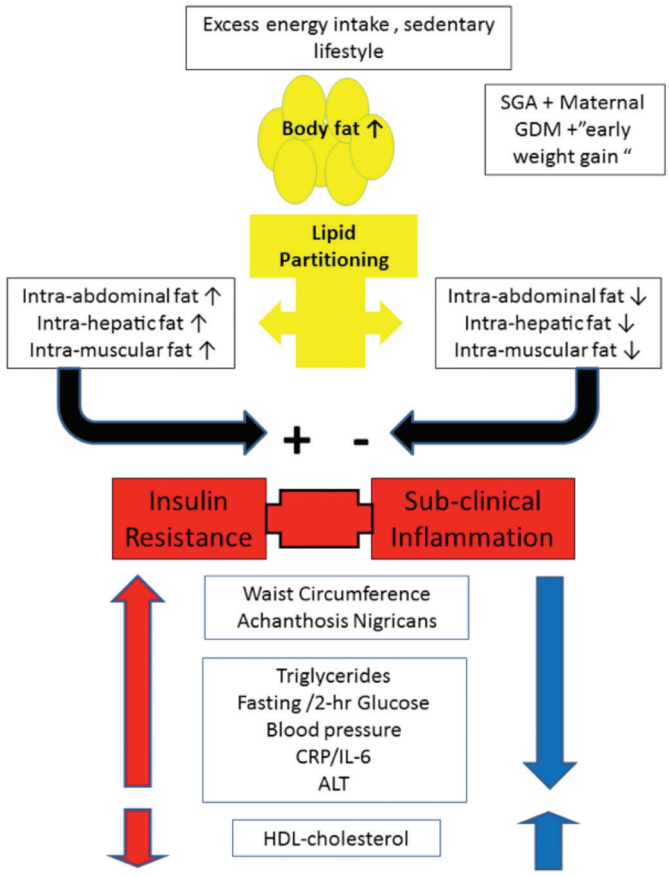

Figure 1. Pathophysiology of insulin resistance and its clinical manifestations.

Energy excess and a sedentary lifestyle lead to increased body fat. The ability of subcutaneous fat tissue to expand will determine the lipid partitioning profile. Those with greater ability to expand their subcutaneous depot will have less intra-abdominal and liver/muscle fat deposition and will thus be more insulin sensitive. Those with inability to increase subcutaneous fat will have an unfavorable lipid partitioning profile with increased intra abdominal lipid deposition as evidenced by greater waist circumference and liver/muscle lipid deposition, manifesting as greater insulin resistance and a pro inflammatory profile. This adverse profile leads to elevation of plasma glucose, triglycerides and blood pressure and reduced high density lipoprotein-cholesterol.

HDL: high density lipoprotein, CRP/IL-6: C-reactive protein/interleukin-6, ALT: alanine amino transferase, SGA: small for gestational age, GDM: gestational diabetes mellitus

syndrome is the International Diabetes Federation definition which provides thresholds for defining the presence of each factor (60). This definition is appropriate for children older than 10 years of age. Most definitions indicate that the presence of a specific number of risk factors, such as obesity and dyslipidemia for example, establishes the diagnosis of Metabolic syndrome. This diagnosis is important as it indicates future health risks for the individual patient.

However, care should be exercised as each component of such definitions should not be used as a dichotomous factor. Rather, the metabolic risk conferred by each factor is continuous and does not have a clear threshold effect. It is well established that beta cell function and peripheral IR are associated with elevated two hour glucose levels, even within the normal glucose tolerance range, without meeting the criteria for IGT (61). Thus, for example, a child with a fasting glucose of 95 mg/dL and a two hour glucose of 135 mg/dL does not meet the criteria for IFG or IGT yet may have substantial impairment of beta cell function (61). Similarly, the degree of obesity is important but the lipid partitioning profile is a stronger determinant of the metabolic profile. Thus, an overweight child on the 93rd centile for BMI, who has an increased waist circumference which is a surrogate of increased intra-abdominal lipid deposition may have a much worse metabolic profile compared to a child with a BMI on the 95th centile with low levels of abdominal fat. Similarly, elevated triglyceride concentration, even below the typical threshold of 150 mg/dL may be associated with significant IR and future metabolic risk (62). Thus, individual factors comprising the clinical definitions of Metabolic syndrome should be used as continuous variables as they are associated with a continuous risk. Each component of the syndrome should be assessed longitudinally for its dynamics in response to any intervention. Each obese child should be evaluated for the presence of individual risk factors and those with more metabolic and anamnestic elements are probably those who should be referred for more intensive interventions. Diagnosis of the Metabolic syndrome in an adult is a clear and established indication of increased cardiovascular risk. Diagnosing the presence of the Metabolic syndrome in a child, regardless of the definition used, should be interpreted with caution. It should indicate to the caregiver that multiple metabolic derangements share a common pathophysiology. This translates from a clinical point of view into avoiding trying to address individual components separately. Rather-improving insulin sensitivity, by means of weight loss, dietary modifications, exercise and/or by pharmacological means, will result in improvement of several factors associated with the Metabolic syndrome in parallel. Moreover, meeting the criteria of the metabolic syndrom implies that the child has CVRFC and is at a greater risk for the development of T2DM and cardiovascular disease at an early age. However, failing to meet these criteria is by no means an indication of "healthy obesity". Rather, it is unclear at present if every element of such definitions has an equal risk implication for the future of the child. Some argue that the obesity component has greater importance while others argue that the presence of IGT has greater impact than other factors. Thus the relative importance of each individual component of the Metabolic syndrome is debatable.

Conclusion

Overweight and obese children and adolescents should be evaluated, keeping in mind that the metabolic morbidity associated with obesity is not necessarily due to the degree of obesity *per se*. Rather, an in depth evaluation into lipid partitioning and a clinical understanding that the presence of cardiovascular risk factors stems from a shared pathophysiology should guide the caregiver in assessing the metabolic risk and tailoring appropriate intervention for each specific child. Figure 1 depicts the pathophysiology and the physical/laboratory manifestations of the IR syndrome in children.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ahmad Ighbariya, Ram Weiss, Concept: Ahmad Ahmad Ighbariya, Ram Weiss, Design: Ahmad Ahmad Ighbariya, Ram Weiss, Data Collection or Processing: Ahmad Ighbariya, Ram Weiss, Analysis or Interpretation: Ahmad Ighbariya, Ram Weiss, Literature Search: Ahmad Ighbariya, Ram Weiss, Writing: Ahmad Ighbariya, Ram Weiss.

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REVIEW

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Current Nomenclature of Pseudohypoparathyroidism: Inactivating Parathyroid Hormone/Parathyroid Hormone-Related Protein Signaling Disorder

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Abstract

Disorders related to parathyroid hormone (PTH) resistance and PTH signaling pathway impairment are historically classified under the term of pseudohypoparathyroidism (PHP). The disease was first described and named by Fuller Albright and colleagues in 1942. Albright hereditary osteodystrophy (AHO) is described as an associated clinical entity with PHP, characterized by brachydactyly, subcutaneous ossifications, round face, short stature and a stocky build. The classification of PHP is further divided into PHP-Ia, pseudo-PHP (pPHP), PHP-Ib, PHP-Ic and PHP-II according to the presence or absence of AHO, together with an *in vivo* response to exogenous PTH and the measurement of Gsα protein activity in peripheral erythrocyte membranes *in vitro*. However, PHP classification fails to differentiate all patients with different clinical and molecular findings for PHP subtypes and classification become more complicated with more recent molecular characterization and new forms having been identified. So far, new classifications have been established by the EuroPHP network to cover all disorders of the *PTH* receptor and its signaling pathway. Inactivating PTH/PTH-related protein signaling disorder (iPPSD) is the new name proposed for a group of these disorders and which can be further divided into subtypes - iPPSD1 to iPPSD6. These are termed, starting from PTH receptor inactivation mutation (Eiken and Blomstrand dysplasia) as iPPSD1, inactivating *Gs*α mutations (PHP-Ia, PHP-Ic and pPHP) as iPPSD2, loss of methylation of *GNAS DMRs* (PHP-Ib) as iPPSD3, *PRKAR1A* mutations (acrodysostosis type 1) as iPPSD4, *PDE4D* mutations (acrodysostosis type 2) as iPPSD5 and *PDE3A* mutations (autosomal dominant hypertension with brachydactyly) as iPPSD6. iPPSDx is reserved for unknown molecular defects and iPPSDn + 1 for new molecular defects which are yet to be described. With these new classifications, the aim is to clarify the borders of each different subtype of disease and make the classification according to molecular pathology. The iPPSD group is designed to be expandable and new classifications will readily fit into it as necessary.

Keywords: Pseudohypoparathyroidism, inactivating parathyroid hormone/parathyroid hormone related protein signaling disorder

Introduction

Pseudohypoparathyroidism (PHP) is a group of rare, related, highly heterogeneous disorders, which are characterized by end-organ resistance to parathyroid hormone (PTH) action. PHP and related disorders are caused by the genetic and/or epigenetic changes leading to down-regulation of a cyclic adenosine monophosphate (cAMP) generator, mostly related to the *GNAS* gene (1,2,3,4,5). *GNAS* is an imprinted gene which gives rise to multiple gene products, including transcripts that encode the *α-subunit of the stimulatory guanine nucleotide-binding protein (G protein)* (*Gs*α), *extra-large Gs*α (*XL*α*s*), and *neuroendocrine secretory protein 55 (NESP55),* as well as to noncoding *A/B* (also referred to as *1A*) and *antisense transcripts (GNAS-AS1)*.

Gsα is a ubiquitously expressed signaling protein having a role in the actions of many hormones and other endogenous molecules through the generation of intracellular cAMP and encoded by *GNAS* exons 1-13 (1,2,3,4,5). Other *GNAS* transcripts *NESP55*, *XL*α*s,* and *A/B*, with the exception of *GNAS-AS1* consists of distinct exons, and all contain their own, differentially methylated, unique first exons (DMRs), which are spliced onto exon 2 of *GNAS*. So all of these transcripts, from exon 2 on, are identical in sequence to *Gs*α (6,7,8,9,10,11). Thus a structural or epigenetic change in other *GNAS* trancripts also affects *Gs*α function.

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Expression patterns of Gsα and other GNAS transcripts in different tissues determine the disease phenotype when *GNAS* mutations are present. The *Gs*α transcript is biallelically expressed in most tissues. However, silenced paternal Gsα expression in some tissues, including proximal renal tubules, neonatal brown adipose tissue, thyroid, gonads, the paraventricular nucleus of the hypothalamus and pituitary can cause hormone resistance in cases of maternal mutations (12,13,14,15,16,17,18). Thus, mutations on maternal alleles cause hormone resistance i.e. PHP.

Historically PHP is the first hormone-resistance syndrome, described by Albright et al (19) and characterized by hypocalcemia, hyperphosphatemia, and elevated PTH levels and Albright hereditary osteodystrophy (AHO). Clinical features of AHO are obesity, round face, short stature, brachydactyly (BD), subcutaneous ossifications and mental retardation. AHO features occur regardless of the parental origin of the *Gs*α mutation, because AHO features are thought to result from *Gs*α haploinsufficiency, primarily in those tissues where *Gs*α expression is biallelic. Consistent with this interpretation, changes in growth plate chondrocytes and subcutaneous ossifications occur, regardless of whether the disrupted allele is inherited from the mother or the father (20,21). Thus, AHO features are seen both in patients with maternal mutations i.e. PHP and paternal mutations, e.g. pseudo-PHP (pPHP), which is characterized by absence of PTH and/or hormonal resistance (Table 1). However, recent data from human studies have revealed that *Gs*α imprinting may be present in some features of AHO, that is obesity and cognitive impairment occur predominantly in patients with PHP (22,23).

Pseudohypoparathyroidism Classification

PHP is subdivided into type I and type II. Type I is defined as the failure to increase both urinary cAMP and urinary phosphate excretion in response to exogenous PTH administration (1,2,3,4,5,24). In PHP-II, urinary cAMP generation in response to exogenous PTH administration is normal, but the urinary excretion of phosphate is impaired (25). Although the common biochemical features of PTH resistance are hypocalcemia, hyperphosphatemia, and elevated PTH levels, and found in PHP-Ia, PHP-Ic, and PHP-Ib; AHO is the part of clinical picture in PHP-Ia, PHP-Ic, pPHP and occasionally in PHP-Ib. In PHP-Ia/PHP-Ic, in addition to PTH resistance, hypothyroidism, growth hormone deficiency and hypogonadism are also demonstrable reflecting target-organ resistance to thyroid-stimulating hormone (TSH), growth hormone-releasing hormone (GHRH) and gonadotropins, respectively (1,2,3,4,5).

This complex classification of PHP is based on several distinct criteria, including the presence of AHO features, hormone resistance, urinary cAMP and phosphaturic response to exogenous PTH and *Gs*α activity (Table 1). However, there are some combinations of features which do not fit readily into this classification, especially with recent development in the field.

Controversies in Pseudohypoparathyroidism Type I

The presence or absence of hormonal resistance is the one of the key findings, which differentiates PHP from pPHP, maternal from paternal mutations, respectively. However, mild resistance to PTH and possibly to other hormones such as TSH, has been described in patients carrying a paternal *GNAS* mutation, that is patients with pPHP (26), so that hormonal resistance is now not only associated with PHP, but with pPHP as well.

Another cornerstone of the earlier classification of PHP is presence or absence of features of AHO, which differentiates PHP-Ia/PHP-Ic from PHPI-b. However, a number of reports from the last decade have also shown that AHO features can exist in patients with epigenetic abnormalities of *GNAS* or namely PHP-Ib (27,28,29,30). Furthermore, *GNAS* methylation changes reminiscent of PHP-Ib have been reported in PHP-Ia patients with GNAS deletions (31). These findings suggest a molecular and clinical overlap between the two subtypes.

The measurement of Gsα protein activity from erythrocyte membranes is one diagnostic method used for differentiating PHP-Ic from PHP-Ia/pPHP, in patients with AHO features and carrying *GNAS* coding mutations. Additionally, according to the previous criteria, Gsα activity is expected to be normal in patients with PHP-Ib (1,2,3,4,5,6). However, recently PHP-Ib patients have been shown to have a moderate reduction in Gsα activity, in a similar but less severe manifestation as patients with PHP-Ia/pPHP (32). Thus, PHP-Ib patients having methylation abnormalities and with AHO features might also have low Gsα activity and the clinical and biochemical findings of these patients are consistent with PHP-Ia (32). On the other hand, if Gsα activity is normal in the patient with PHP-Ib and AHO features, the patients could be described as PHP-Ic, clinically and biochemically (27,28,29,30,32).

Additionally, molecular defects are not unique to PHP-Ic. The loss-of-function mutations in the carboxyl-terminus of *GNAS,* causing disruption of receptor-mediated activation but conservation of adenylyl cyclase receptor-independent activation, lead to PHP-Ic (33,34,35). And methylation defects, as found in PHP-Ib could be another molecular defect present in patients described clinically and biochemically as PHP-Ic (34).

Table 1. Disease related parathyroid hormone/parathyroid hormone-related protein and cyclic adenosine monophosphate signaling pathway and former classification according to clinical features and molecular defects

	Molecular defects	Parental origin	Hormonal abnormalities	Additional clinical features	Urinary cAMP to exogenous PTH	Urinary phosphate to exogenous PTH	Erythrocyte Gsα activity
PHP Ia (OMIM #103580)	*Gs*α coding mutations-inactivating	Maternal	PTH resistance TSH resistance Other hormone resistances (GHRH, gonadotrophins, calcitonin, etc.)	AHO features	Blunted	Blunted	Reduced
PHP Ic (OMIM #612462)	*Gs*α coding mutations-inactivating	Maternal	PTH resistance TSH resistance Other hormone resistances (GHRH, gonadotrophins, calcitonin, etc.)	AHO features	Blunted	Blunted	Normal
pPHP (OMIM #612463)	*Gs*α coding mutations-inactivating	Paternal	No	AHO features	Normal	Normal	Reduced
POH (OMIM #166350)	*Gs*α coding mutations-inactivating	Paternal	No	No	Normal	Normal	Reduced
PHP Ib (OMIM #603233)	Methylation defects	Maternal	PTH resistance TSH resistance	No	Blunted	Blunted	Normal
Acrodysostsosis type 1 (OMIM #101800)	*PRKAR1A* mutations-leading reduced PKA activity	Autosomal dominant	PTH resistance TSH resistance in some	AHO Typical face	Normal	Blunted	Normal
Acrodysostsosis type 2	*PDE4D* mutations-activating	Autosomal dominant	PTH resistance TSH resistance Other hormone resistances (GHRH, gonadotrophins, calcitonin, etc.)	AHO Typical face	Normal	Blunted	Normal
Hypertension and brachydactyly Syndrome (OMIM #112410)	*PDE3A* mutations-activating	Autosomal dominant	Unknown	AHO Hypertension	Unknown	Unknown	Unknown
Blomstrand chondrodysplasia (OMIM #215045)	*PTH1R* mutations-inactivating	Autosomal recessive	Unknown	Severe skeletal dysplasia, Lethal, abnormal breast and tooth development, Accelerated ossification	Unknown	Unknown	Unknown
Eiken syndrome (OMIM #600002)	*PTH1R* mutations-inactivating	Autosomal recessive	PTH resistance (mild)	Severe skeletal dysplasia, dwarfism, Retarded ossification	Unknown	Unknown	Unknown

OMIM: Online Mendelian Inheritance in Man, PHP: pseudohypoparathyroidism, pPHP: pseudo-pseudohypoparathyroidism, POH: progressive osseous heteroplasia, cAMP: cyclic adenosine monophosphate, PTH: parathyroid hormone, AHO: albright hereditary osteodystrophy, TSH: thyroid-stimulating hormone, GHRH: growth hormone-releasing hormone, Gsα: α-subunit of the stimulatory guanine nucleotide-binding protein, PKA: protein kinase A

There are too many inconsistencies described in the literature of PHP-I subtypes, both clinically, genetically and biochemically when using the earlier classification so that a newer, comprehensive classification would be welcome.

Furthermore, progressive osseous heteroplasia (POH) is a distinct entity described in patients with paternally inherited *GNAS* mutations, usually causing truncation of the gene product (36). Features typical of AHO and hormone resistance have been detected in some patients with POH. Conversely, some PHP-Ia patients with maternal mutations present with POH-like progressive deepening of the heterotopic ossifications (37,38). Furthermore, POH lesions show a mosaic distribution and follow dermomyotomes, usually with a unilateral pattern. Experimental evidence has shown that a loss of heterozygosity at the *GNAS* locus, with somatic mutations in a progenitor cell of somitic origin, may cause severe, progressive heterotopic ossifications that show a similar unilateral distribution (39).

Controversies in Pseudohypoparathyroidism Type II

The differentiation of PHP-I from PHP-II is made by comparing the *in vivo* response to exogenous PTH in terms of nephrogenic cAMP synthesis and phosphaturia. The presence of cAMP elevation without phosphaturia marks PHP-II (24,25). Until 2011 no clear etiopathogenesis had been described for PHP-II (40). However, then and since, patients with acrodysostosis, have been found to exhibit biochemical abnormalities found in PHP-II. In addition, heterozygous mutations in *PRKAR1A,* which encodes the regulatory subunit of protein kinase A (PKA) and *PDE4D*, which encodes phosphodiesterase type 4, have been found in patients with acrodysostosis (40,41,42). Both *PRKAR1A* and *PDE4D* have a role in cAMP generation, down stream of Gsα. Thus, a heterogeneous group of rare diseases, characterized by skeletal dysplasia, has been included in the classification of PHP.

Acrodysostosis is characterized by skeletal dysplasia and has characteristic features, including BD, facial dysmorphism and, in some cases, mental retardation (43,44,45,46,47). Hormone resistances, usually PTH and/or TSH resistance, have been detected in about 60-70% of acrodysostosis patients with a *PRKAR1A* mutation and in 10-20% of cases with *PDE4D* mutations. However, typical facial features and more generalized BD distinguishes acrodysostosis from PHP (46,48). On the other hand, it has been shown that some cases with a phenotype typical of PHP-Ia also have *PRKAR1A* mutations (49,50).

Another disease that has been shown to involve the cAMP pathway is hypertension and brachydactyly syndrome (HTNB-Bilginturan syndrome, OMIM #112410) which is characterized by hypertension, BD type E (BDE) and short stature. Heterozygous mutations in *PDE3A* have been identified in patients affected with HTNB (51). Of note, BDE and short stature are clinical features of AHO.

Although these two diseases, acrodysostosis and HTNB syndrome exhibit molecular defects in the PTH-cAMP pathway and are clinically identical to PHP/pPHP, they were not previously included in the classification of PHP. Furthermore, disorders associated with an impaired function of *PTH1R*, i.e. Blomstrand and Eiken skeletal dysplasia, are also currently not included in the classification of PHP. In addition, other diseases featuring defects in cAMP and its downstream pathway, should have a place in the classification if they are described in the future.

Rationale for the New Classification

In light of this new evidence the EuroPHP network, which is composed of experts from different independent centres, proposed a new classification to create a uniform terminology and classification based on the current knowledge of PHP (52). The term "inactivating PTH/PTHrP signalling disorder" (iPPSD) was selected since it describes the common mechanism responsible for the diseases, encompasses all disorders related to this pathway and was flexible enough to incorporate new development in this field (52).

The terms "PHP" and "pPHP" are confusing, both for description of the diseases and for use in communication. iPPSD is more compact and describes a group of disorders which makes the disease classification easier from the beginning. For the diagnosis of iPPSD, major and minor criteria have been described and a minimum of one of the major criteria is mandatory for clinical diagnosis of iPPSD (see Table 2) (52). PTH resistance or ectopic ossifications could be diagnostic for iPPSD with or without the presence of minor criteria. However, since BDE is a common feature of several other diseases and syndromes, in patients exhibiting BDE at least one major or two minor criteria should also be present for a diagnosis of iPPSD.

The entities included in iPPSD classification, with known molecular causes of impaired PTH/parathyroid hormone-related protein (PTHrP) signaling (52) are:

- Inactivating mutations of *PTH1R*

- Heterozygous inactivating mutations in the coding sequence of *GNAS*-Gsα

- Methylation changes of the DMRs of *GNAS* caused by deletions or duplications (*STX16; NESP; GNAS*-AS1) or paternal UPD of chromosome 20q or unknown mechanism(s)

- Heterozygous mutations of *PRKAR1A* leading to reduced PKA activity

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- Heterozygous activating mutations of *PDE4D*

- Heterozygous activating mutations of *PDE3A*

Major and Minor Criteria

Major Criteria

1. PTH resistance: PTH resistance is defined as elevated PTH with or without hypocalcemia, hyperphosphatemia. Resistance occurs only at the renal proximal tubule and distal renal tubule and PTH is functionally intact and therefore, the patients will have hypocalciuria (1,2,3,4,5).

For evaluation of PTH resistance and to differentiate PTH resistance from normocalcaemic hyperparathyroidism, renal failure, vitamin D deficiency and any form of secondary hyperparathyroidism, the following laboratory tests should be performed; ionized calcium, total calcium, phosphate, magnesium, PTH, vitamin D (25-hydroxyvitamin D), creatinine, urinary calcium and urinary phosphate excretion. A PTH infusion test is reserved for challenging cases (1,2,3,4,5,52).

2. Ectopic ossification: Ectopic ossifications are foci of bone formation in the adipose or dermal tissue, which manifest as superficial, subcutaneous nodules (1,2,3,4,5). Progression of heterotopic osseous calcifications, usually from the dermal and subcutaneous tissues to the deeper tissues, such as muscles and tendons may be seen and defined as POH (36,37,38). In children, ectopic ossifications are highly suggestive of an inactivating *GNAS* mutation, i.e. iPPSD (52).

Table 2. Diagnosis of inactivating parathyroid hormone/ parathyroid hormone-related protein signalling disorder with major and minor criteria

1. Major criteria
1. PTH resistance
2. Ectopic ossification
3. Brachydactyly type E
2. Minor criteria
1. TSH resistance
2. Other hormonal resistances
3. Motor and cognitive retardation or impairment
4. Intrauterine and postnatal growth retardation
5. Obesity/overweight
6. Flat nasal bridge and/or maxillar hypoplasia and/or round face

Parathyroid hormone/parathyroid hormone-related protein signalling disorder clinical diagnosis: Either presence of one major criteria, either number 1 or 2; or presence of major criteria number 3 and at least 2 minor criteria

PTH: parathyroid hormone, TSH: thyroid-stimulating hormone

Diagnosis of ectopic calcification can be made by inspection and palpation on physical examination and may be detected by X-ray imaging if tissue is large enough. In selected cases, diagnosis may involve biopsy, but it is not recommended due to an increased risk for progression of biopsied osseous tissue (52). Fibrodysplasia ossificans progressiva (OMIM #135100) and post-traumatic osteoma cutis should be differentiated (53). Calcification rather than ossification should be considered as a differential diagnosis, as in tumoral calcinosis which is related to the defective activity of fibroblast growth factor 23 (FGF23), in which mutations in *FGF23, GALNT3* and *α-klotho* have been identified (54).

3. BDE: BD refers to shortening of the fingers, toes or both. BD in iPPSD should be classified as BDE (OMIM #113300), which is characterized by variable shortening of the metacarpals, with more or less normal length of phalanges, occasionally accompanying shortened metatarsals (55). Hypoplastic and partially fused metacarpal epiphyses, seen on radiographs, are the cause of BD and lead to BDE. In addition, the terminal phalanges are often short (55). It can either present in isolation or as part of a genetic disorder, most of which are included in the iPPSD classification (56).

Almost all patients with *GNAS* mutations have BD and decreased Gsα activity, which is usually decreased by around 50% (57,58,59). Although, Gsα activity is supposed to be normal in cases with methylation abnormalities such as in the entity known as PHP-Ib formerly, PHP-Ib patients with an AHO phenotype have more severely diminished Gsα activity levels than those who do not have the AHO phenotype (32). Furthermore, BD has been detected in both patients with a genetic mutation and in those with an imprinting error in PHP-Ib but at differing median ages of detection; 7.2 years in the former and 13.2 years in the latter (60). These results could be related to the degree of the Gsα functional impairment with a more severe loss of function leading to earlier BD development. It can be difficult to detect BD, especially in early childhood, and tends to become more evident during early puberty. BD can be overlooked when all bones are short as in acrodysostosis which has affected the patient since early childhood (61).

Clinical and radiological evaluation of hand bones are necessary for a diagnosis of BDE. On clinical examination, by using a straight ruler at the head of the metacarpals of the closed fist, the tips of 3rd, 4th and 5th metacarpals should be in a line and touching the ruler. If the 4th or 5th metacarpals are receding, this can be accepted as a positive metacarpal sign, also known as Archibald's sign (55,62,63). The evaluation on X-rays can be done in a similar fashion (55,63). However, normally this sign is positive in only 9.6% of individuals and if a deviation of more than 2

mm is accepted as a limit, only 0.5% of individuals have the sign (64). In addition, if all bones are short, this metacarpal sign will be negative. If so, each metacarpal and phalangeal bone should be measured and evaluated separately (metacarpo-phalangeal profile). If shorter than 2 standard deviation scores (SDS) for the individual bone, it is accepted as short and BD (65). Differential diagnoses for BDE are Turner syndrome, tricho-rhino-phalangeal syndrome (TRPS) including TRPS type I, (OMIM #190350), TRPS type II (OMIM #150230) and TRPS type III, (OMIM #190351), BDE with short stature, parathyroid hormone-like hormone (PTHLH, OMIM #613382), isolated BDE: HOXD13 type (OMIM #113300) and BD mental retardation syndrome (OMIM #600430) (56).

While existence of PTH resistance or ectopic ossifications are considered diagnostic for iPPSD as major criteria; BD is less specific and should, therefore, be present with at least one other major or two minor criteria to consider the diagnosis of iPPSD.

Minor Criteria

1. Thyroid-Stimulating Hormone Resistance

TSH resistance is usually characterized by mildly elevated TSH levels with a normal or low-normal free thyroxine (T4) level. TSH levels are usually below 50 mIU/L (66,67). Sometimes patients present with clinical symptoms of hypothyroidism, such as prolonged jaundice, macroglossia, hypothermia and umbilical hernia in neonates or constipation and listlessness in infants (66,68).

Hypothyroidism occurs in the absence of goiter and markers of autoimmune disease (66,67). In laboratory evaluation, TSH, free-T4, anti-thyroid antibodies and thyroid ultrasound should be performed. TSH receptor inactivation mutation can be considered in the differential diagnosis (52,66,67).

TSH resistance could be a first manifestation of iPPSD, especially if referred from the neonatal screening program for congenital hypothyroidism (68,69).

2. Other Hormone Resistances

Other hormone resistances are also present in iPPSD. Growth hormone deficiency due to resistance to GHRH, is the next most frequent resistance reported, and found in 60% of patients with PHP-Ia (70,71,72). Calcitonin resistance has also been also described in patients with PHP-Ia, but with no known associated clinical or biochemical abnormalities (67). Gonadotropin resistance, with elevated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, is a further G-protein coupled hormone resistance reported in iPPSD (73,74). Glucagon and adrenaline resistances have been demonstrated through *in vivo* testing in patients with low Gsα bioactivity (75,76).

For evaluation of growth hormone deficiency; insulin-like growth factor (IGF)-1, IGFB-3 and growth hormone stimulation tests can be performed, if necessary. Serum measurements of calcitonin, LH and FSH are helpful if the respective resistance is suspected and in addition a gonadotropin-releasing hormone/LH-releasing hormone test may be performed.

Motor and Cognitive Retardation or Impairment

Psychomotor and cognitive impairments have been described as a feature of AHO. A significant proportion of patients (40-70%) with a maternal coding mutation of *GNAS,* (formerly PHP-Ia) has been shown to have cognitive impairment (22,77). However, cognitive impairment is seen rarely in patients with paternally inherited *GNAS* mutations (PPHP, POH) ranging from 0% to 10% of cases (78). The patients with methylation abnormalities, i.e. PHP-Ib, may also have cognitive impairment (79,80,81) especially if they have AHO features, as cognitive impairment is reported in almost half of them (30). Additionally, varying severity of psychomotor and cognitive impairment has been described in some patients with acrodysostosis (42,44,45). It has been suggested that psychiatric disorders may be part of the disease spectrum (82). However, patients with paternal mutations of *GNAS* or epigenetic modifications of *GNAS* DMRs seem to be unaffected (22,83).

Intrauterine and Postnatal Growth Retardation

Intrauterine growth retardation (IUGR) has been frequently observed in patients with inactivating *GNAS* coding mutations. Although both paternal and maternal inherited mutations are associated with IUGR, patients harbouring mutations on the paternal *GNAS* allele are more severely affected, especially when the mutation is in exons 2 to 13, compared with patients with *GNAS* exon 1/intron 1 mutations (84). The reason for paternal GNAS exon 2-13 mutations causing more severe IUGR is due to an impairment of another transcript of *GNAS*, *XLαs*, which is essential for early postnatal adaptation to feeding and survival, as well as glucose counterregulation (85,86). IUGR has also been described in other iPPSD, such as acrodysostosis with mutations in *PRKAR1A* or *PDE4D*, and in patients with mutations in *PDE3A* (40,41,49,51). However, loss of methylation at the maternal *GNAS A/B*: PHP-1b has been associated with increased intrauterine growth and high birth weight (87).

Postnatal growth retardation resulting in short final height is a common finding in PHP-Ia and acrodysostosis. Growth hormone deficiency and premature closure of the epiphysis are the causes of short stature (40,41,70,88). Rarely, growth retardation has also been described in PHP-Ib (27,30) and in patients with Eiken dysplasia (89).

Obesity/Overweight

Obesity or overweight is commonly present but, is possibly the most nonspecific minor sign of iPPSD. However, early onset obesity is an important clinical feature manifesting from the first few months of life and resulting in severe obesity during infancy. However, obesity tends to improve as the patient ages. In adulthood, only about two thirds of PHP-Ia are obese with a mean body mass index (BMI) Z-score of 1.7 ± 0.2 (77,90,91).

Patients with maternally inherited GNAS coding exon mutations, but not those carrying mutations on the paternal allele, have obesity/overweight. This may be helpful in differentiating PHP-Ia from pPHP. Growth hormone deficiency, impaired lipolytic response to adrenaline (76) or decreased resting energy expenditure (92) may all contribute to the development of obesity in patients with mutations on the maternal allele (23,91). Obesity is also a frequent feature in patients affected with acrodysostosis (40,49,93). For evaluation, weight charts and BMI SDS or percentile charts are necessary. Monogenic obesity stemming from leptin/melanocortin pathway abnormalities should be considered in differential diagnosis of early onset obesity (94).

Flat Nasal Bridge and/or Maxillar Hypoplasia and/or Round Face

Patients with acrodysostosis have typical facial features with flat nasal bridge and/or maxillar hypoplasia and patients with PHP-Ia have a round face which is inconsistent with the degree of obesity. These findings are, however, nonspecific (19,45).

The New Classification (Figure 1)

The former classification of PHP/pPHP is based on the clinical and biochemical phenotype. However, a new classification, iPPSD, has been identified according to described clinical and biochemically criteria. Further subtyping will be possible by identifying the underlying molecular genetic or epigenetic defect. Thus, the term iPPSD refers to the pathophysiology, which is impairment of PTH/PTHrP signaling, and the number refers to the underlying molecular defect as shown below (52).

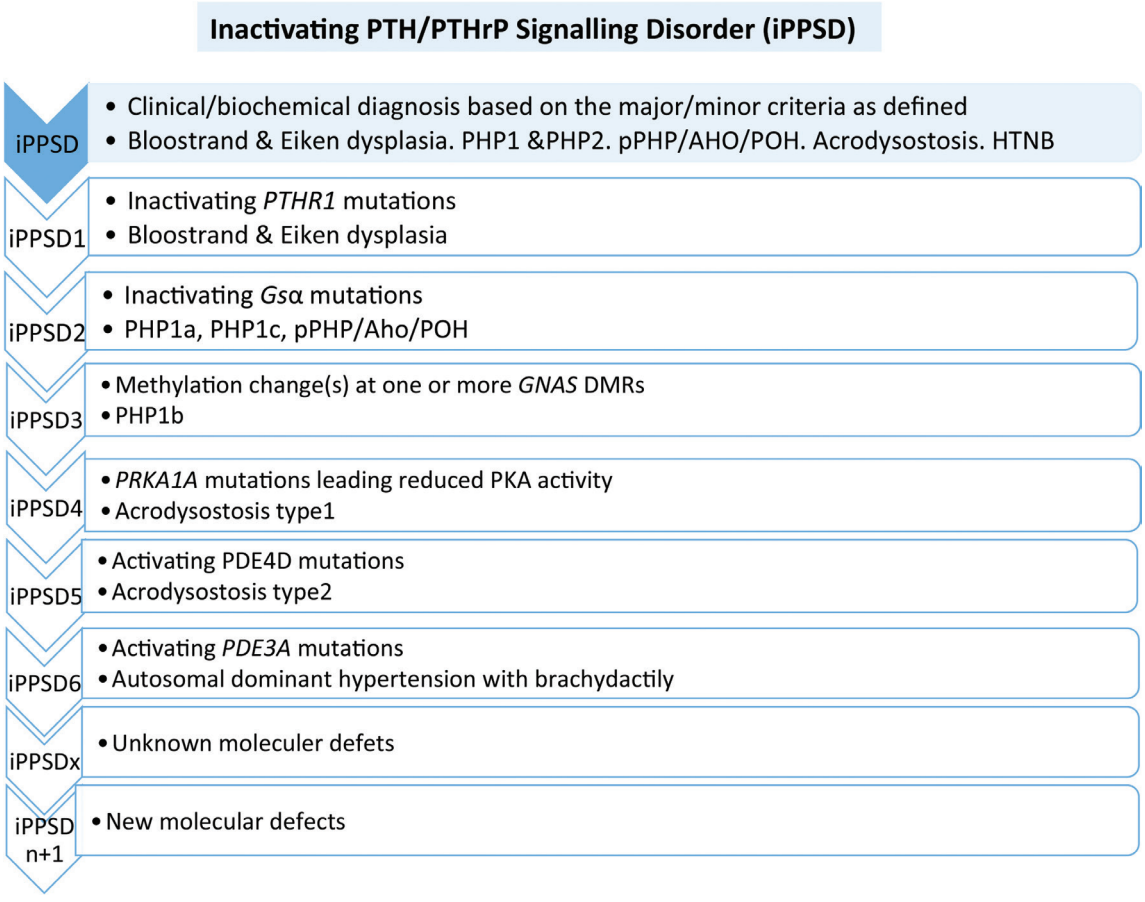

Figure 1. The new classification proposed by the European Pseudohypoparathyroidism Network (52) with new nomenclature on the left with molecular defects and the disease names listed in the right column

PTH: parathyroid hormone, PTHrP: parathyroid hormone-related protein, iPPSD: inactivating parathyroid hormone/parathyroid hormone-related protein signaling disorder, DMRs: differentially methylated regions, POH: progressive osseous heteroplasia, PHP: pseudohypoparathyroidism, pPHP: pseudopseudohypoparathyroidism, AHO: Albright hereditary osteodystrophy, PKA: protein kinase A, HTNB: hypertension and brachydactyly syndrome

The Classification of Inactivating PTH/PTHrP Signalling Disorder (52)

iPPSD: Clinical/biochemical diagnosis based on the major/minor criteria described, without any genetic investigation/diagnosis.

iPPSD1: Loss-of-function mutation in *PTH1R*.

iPPSD2: Loss-of-function mutation in *Gs*α.

iPPSD3: Methylation change(s) at one or more *GNAS* DMRs, associated with or without a genetic deletion (*STX16, NESP55, AS* etc.) or cytogenetic (UPD) defect. The loss of methylation at the *GNAS A/B* is the common mechanism shared by these patients.

iPPSD4: Mutation in *PRKAR1A* leading reduced PKA activity.

iPPSD5: Gain-of-function mutation in *PDE4D* mutation.

iPPSD6: Gain-of-function mutation in *PDE3A* mutation.

iPPSDx: Absence of any genetic/epigenetic defect after molecular investigations of known genes described above but fitting the criteria for iPPSD.

iPPSDn + 1: Identification of a new gene and/or molecular defect will increment the number of iPPSD types by one, i.e. iPPSD7, iPPSD8 and so on.

With this new classification, the disorders were stratified according to etiopathogenesis, thus mechanism and simplified the concept of the overlapping disorders under a single umbrella. Additionally, it is flexible enough to accommodate new defects which may be discovered in the future. However, with this classification, the parental origin of the genetic/epigenetic defect is not taken into account, although iPPSD2 and iPPSD3 are imprinting disorders and their clinical presentation depends on the parental origin of inheritance. Although multiple hormone resistance, including PTH resistance, are largely associated with maternal *GNAS* mutations and isolated AHO and/or POH are more often associated with paternal *GNAS* mutations, hormone resistance and POH may be seen in both maternal and paternal inactivating *GNAS* mutations. Therefore, the new classification does not include parental origin of mutation but for genetic counseling this point should be considered. The mechanism of the two allelic *GNAS* mutations can be considered alike. Another point of this classification is the inability to sub-classify individuals with purely clinical findings-molecular analysis is mandatory. Cases should be classified as iPPSD, not iPPSDx, pending definitive molecular diagnosis.

Furthermore, PTHR1 has been included in the classification. However, two main ligands of PTHR1, PTH and PTHrP and related disorders are not chosen as a part of classification. Since, BDE with short stature seen in patients with *PTHLH* mutations, encoding PTHrP, (95,96), this point could be argued. Since these disorders are not primarily related to the signaling pathway defect, it is not included in the definition of main classification.

Conclusion

A new classification has been established by the EuroPHP network to cover all disorders of the PTH receptor and its signaling pathway. iPPSD is the new name proposed for this group of conditions and which are further divided into the subtypes from iPPSD1 to iPPSD6. With this new classification, it is aimed to clarify the border of each different subtype of disease and make the classification according to molecular pathology. The iPPSD group is a growing group of conditions and new entities can readily be fitted into this classification.

Ethics

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REVIEW

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Congenital Hyperinsulinism: Diagnosis and Treatment Update

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Abstract

Pancreatic β-cells are finely tuned to secrete insulin so that plasma glucose levels are maintained within a narrow physiological range (3.5-5.5 mmol/L). Hyperinsulinaemic hypoglycaemia (HH) is the inappropriate secretion of insulin in the presence of low plasma glucose levels and leads to severe and persistent hypoglycaemia in neonates and children. Mutations in 12 different key genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, UCP2, HNF4A, HNF1A, HK1, PGM1* and *PMM2*) that are involved in the regulation of insulin secretion from pancreatic β-cells have been described to be responsible for the underlying molecular mechanisms leading to congenital HH. In HH due to the inhibitory effect of insulin on lipolysis and ketogenesis there is suppressed ketone body formation in the presence of hypoglycaemia thus leading to increased risk of hypoglycaemic brain injury. Therefore, a prompt diagnosis and immediate management of HH is essential to avoid hypoglycaemic brain injury and long-term neurological complications in children. Advances in molecular genetics, imaging techniques (^{18}F-DOPA positron emission tomography/computed tomography scanning), medical therapy and surgical advances (laparoscopic and open pancreatectomy) have changed the management and improved the outcome of patients with HH. This review article provides an overview to the background, clinical presentation, diagnosis, molecular genetics and therapy in children with different forms of HH.

Keywords: Hyperinsulinaemic hypoglycaemia, congenital hyperinsulinaemia, children, diffuse congenital hyperinsulinism, focal congenital hyperinsulinism, sirolimus

Introduction

Hyperinsulinaemic hypoglycaemia (HH), refers to a clinically, genetically and morphologically heterogeneous group of disorders associated with dysregulated insulin secretion. It is the most common cause of persistent hypoketotic hypoglycaemia in neonates and infants and is associated with a significant risk of permanent brain damage. Therefore, it is essential to make a prompt diagnosis and institute immediate management to prevent complications such as epilepsy, cerebral palsy and neurodevelopemental deficits (1).

The metabolic action of insulin on glucose and fuel metabolism increases the risk of neurological injury. Insulin decreases blood glucose level by increasing its peripheral consumption, stimulates glycogen synthesis and inhibits glycogenolysis and gluconeogenesis. On the other hand, insulin has an anabolic effect on fat tissues. It stimulates lipogenesis, inhibits free fatty acid release, and their beta-oxidation and thus inhibits ketone body formation. This accounts for the hypoketotic state, decreasing the availability of alternative fuels for cerebral metabolism (2). As the brain of neonates and infants has a higher rate of glucose comsumption compared to adult subjects, it is more vulnerable to hypoglycaemic brain injury. HH typically presents in the newborn period with severe hypoglycaemia but can also present in infancy, childhood and even as late as adulthood with variable severity and etiology (3,4).

HH can be transient due to certain risk factors, such as birth asphyxia, intra-uterine growth retardation, maternal diabetes mellitus (5), or associated with various overgrowth syndromes like Beckwith-Wiedemann syndrome or metabolic conditions such as congenital disorders of glycosylation (6). Genetic forms of HH congenital hyperinsulinism (CHI) are due to mutation in the genes involved in the regulation of insulin secretion. HH typically presents with fasting hypoglycemia but can present with postprandial hypoglycaemia or in some

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cases hypoglycaemia can be provoked by protein/leucine loading or even exercise. Patients with HH can vary in their presentation from having no symptoms to having severe, medically unresponsive disease which might require a near total pancreatectomy (7).

Histologically, CHI is classified into three subgroups: diffuse, focal and atypical forms (8,9). Diffuse disease affects all the islets in the pancreas, whereas in focal disease the abnormality is confined to a small region of the pancreas. Atypical histological forms of CHI have recently been described (10). Although all the histological subtypes are clinically and biochemically indistinguishable, their differentiation at the histological level is important from the point of the view of management. Recent advances in imaging techniques using ^{18}F-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography/computed tomography (PET/CT) have fundamentally changed management strategies, particularly in patients with focal CHI (11,12).

Mutations in key genes which are involved in the regulation of insulin secretion from pancreatic β-cells underlie the molecular basis of CHI. Until recently mutations in only 12 different genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF4A, HNF1A, HK1, PGM1* and *PMM2*) that lead to dysregulated secretion of insulin had been described (6,13,14,15,16,17,18). More recently there have been single case reports of potentially novel genetic mechanisms of HH associated with other syndromic features (19,20). In the vast majority of patients who are diazoxide responsive, the genetic basis of HH is still not known. This review aims to give an overview of the biochemical and molecular basis of CHI with a focus on describing the latest advances in the diagnosis and treatment of this complex condition.

Physiological Mechanisms Regulating Insulin Secretion from Pancreatic β-cells in Congenital Hyperinsulinism

During the intrauterine period the fetus receives glucose across the placenta via facilitated diffusion. After birth, in term healthy newborns with no risk factors for hypoglycemia, plasma glucose levels tend to shows a sharp decline during the first 24-48 hours, but will then normalize to values around 3.5-5.5 mmol/L. This maintenance of a normal plasma glucose concentration requires an adequate supply of exogenous glucose, endogenous fat, glycogen and potential gluconeogenic substrates (e.g. amino acids, glycerol and lactate). In addition, a functional endocrine system that integrates and modulates substrate mobilization, interconversion and utilization is important, as are the key enzymes involved in glycogen synthesis/glycogenolysis, glycolysis, gluconeogenesis, lipolysis and ketogenesis.

The pancreatic β-cells possesses a signal transduction system, whereby fuel metabolism is intricately linked to regulated insulin secretion (21). Glucose is the most important fuel involved in this so called stimulus-response coupling mechanism. This stimulus response-coupling event is controlled by adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}) located in the pancreatic β-cells membrane (22). Glucose enters the β-cells through facilitative glucose transporters, particularly glucose transporter 2 (GLUT 2) and is converted to glucose-6-phosphate by the enzyme *glucokinase (GCK)* (23). GLUT 2 has high affinity for glucose which allows glucose transport in proportion to the plasma glucose concentration (24).

Glycolysis generates high energy molecules such as ATP and this leads to an increase in the ratio of ATP/adenosine diphosphate (ADP) resulting in the closure of the ATP-K_{ATP}. The inwardly rectifying potassium (Kir6.2) subunit of the K_{ATP} channels are responsible for trafficking of intracellular and extracellular ion exchange, thus maintaining a steady state membrane potential. The closure of the K_{ATP} channels results in depolarization of pancreatic β-cells membranes and activation of intramembraneous voltage-gated calcium channels. Calcium enters into β-cells through these voltage-gated calcium channels and an increase in intracellular calcium triggers secretory granule exocytosis and insulin release (Figure 1).

GCK plays a critical role in acting as a gluco-sensor, providing a link between the extracellular plasma glucose concentration and the metabolism of glucose in β-cells (25). When the plasma glucose concentration is increased, the activity of *GCK* is also increased, hence increasing insulin secretion from the β-cells (Figure 1). Conversely, as the plasma glucose concentration decreases, insulin secretion decreases and serum insulin becomes undetectable when the plasma glucose level is below 3 mmol/L (26,27).

Clinical Presentation and Biochemical Diagnosis of Hyperinsulinaemic Hypoglycaemia

Patients with HH can present with a wide range of symptoms ranging from non-specific adrenergic symptoms (poor feeding, hunger, palpitations, sweating) to life-threatening, neuroglycopenic symptoms (seizures, unconsciousness, lethargy, coma and even death) arising from an inadequate supply of glucose to the brain, resulting in impairment of brain function.

HH most commonly presents during the neonatal period, but can also present during infancy, childhood and even adulthood (4,28). The clinical presentation of hypoglycaemia is most severe in the newborn and may be quite subtle in infancy and the childhood period. Therefore the diagnosis might be missed until later in life (29,30,31). There can

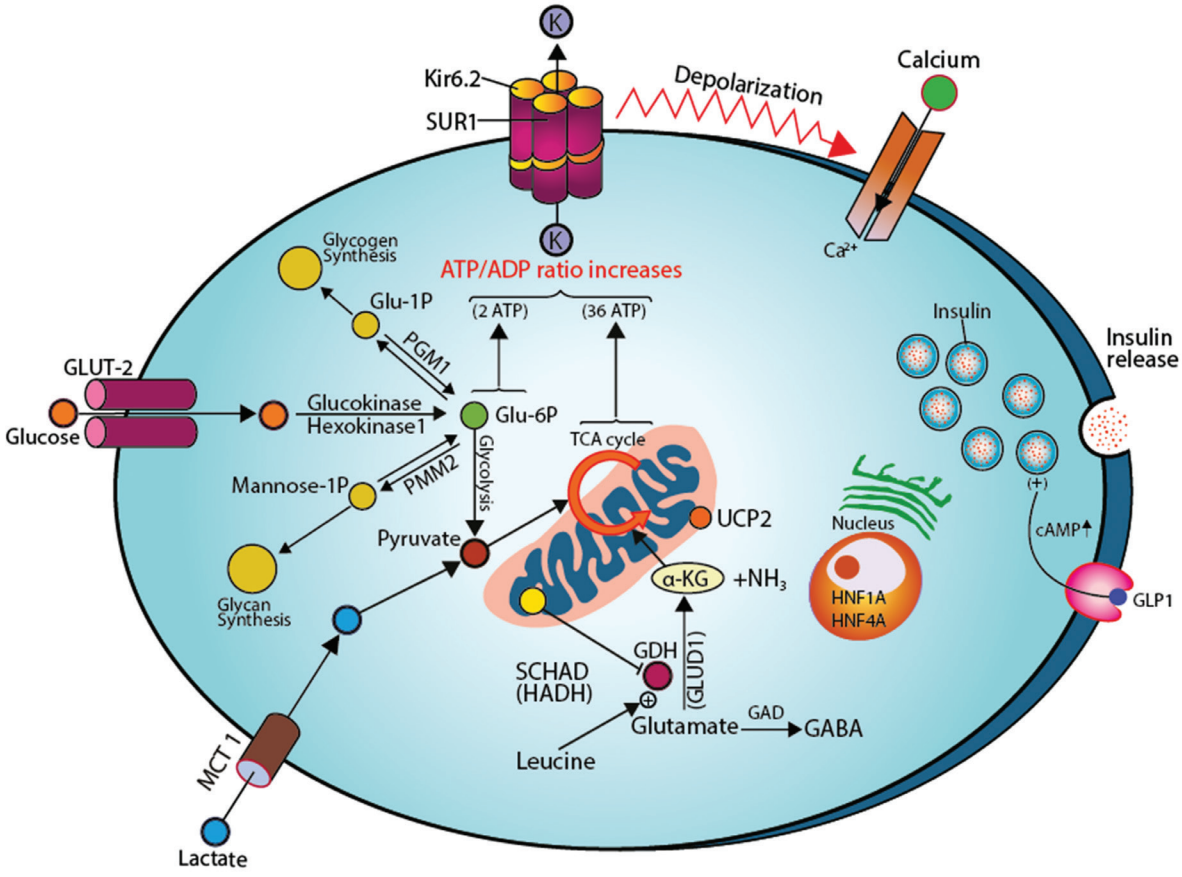

Figure 1. Regulation of insulin release from pancreatic β-cell and sites of gene mutations involved in the genetics etiology of hyperinsulinaemic hypoglycaemia

SUR1: sulphonlyurea receptor 1, Kir6.2: inwardly rectifying potassium channel 6.2, K: potassium, MCT1: monocarboxylate transporter 1, Glu: glucose, P: phosphorus; PGM1: phosphoglucomutase 1, PMM2: phosphomannose-mutase 2, UCP2: mitochondrial uncoupling protein 2, NH_3: ammonia, GDH: glutamate dehydrogenase, *GLUD1: glutamate dehydrogenase 1 gene*, SCHAD: short-chain L-3-hydroxyacyl-CoA dehydrogenase, HADH: hydroxy-acyl-CoA dehydrogenase, HNF1A and 4A: hepatocyte nuclear factor 1A and 4A, Ca^{+2}: calcium; GAD: glutamate decarboxylase enzyme, GABA: γ-aminobutyric acid, GLP1: glucagon like peptide 1, cAMP: cyclic adenosine monophosphate (amplifier for the exocytosis of insulin secreting granule)

be marked phenotypical variability even within the same family.

Newborns with HH may be macrosomic due to intrauterine hyperinsulinaemia. However, the absence of macrosomia does not exclude HH. Hypertrophic cardiomyopathy and hepatomegaly (increased storage of glucose as glycogen) are observed in some patients with HH. The mechanism of cardiomyopathy and hepatomegaly in these patients is unclear but might be related to the effect of foetal hyperinsulinaemia (1).

Early diagnosis of HH is fundamentally important in preventing hypoglycaemic brain injury. Hence, clinicians should always be aware of recognising HH and managing these patients. In any patient with recurrent or persistent hypoglycaemia, HH should be suspected and critical samples at the time of hypoglycaemic episodes should be collected. An intravenous glucose infusion rate requirement of >8 mg/kg/min (normally is 4-6 mg/kg/min) is virtually diagnostic of HH (1). In milder forms of HH, it will be important to establish the duration of fasting and whether the hypoglycaemia is precipitated by meals (protein sensitivity) or by exercise.

Biochemically in HH, there is an inappropriate concentration of serum insulin/c-peptide for the level of plasma glucose (spontaneous or provoked). Low or undetectable serum insulin levels during hypoglycaemia do not exclude the diagnosis of HH (29,30). In some cases serum C-peptide levels (≥0.5 ng/mL) and IGFBP-1 (≤110 ng/mL) may help confirm the diagnosis of HH with specificities of 100% and 96.6%, respectively (29). The metabolic effect of inappropriate insulin secretion is reflected by inappropriately low levels of serum ketone bodies and fatty acids during hypoglycaemic episodes. There is no correlation between measured serum insulin concentration and the severity of the hypoglycaemia

(31). In some difficult cases, the diagnosis of HH should not be based on an isolated serum insulin/c-peptide concentration but on the clinical presentation and the biochemical profiles of insulin action (low β-hydroxybutyrate and fatty acid concentrations). The diagnostic criteria for HH are summarized in Table 1 (29,32,33).

In some instances certain biochemical and clinical features may help in the diagnosis of specific forms of CHI. An elevated serum ammonia concentration in a patient with HH is suggestive of the hyperinsulinism and hyperammonaemia (HI/HA) syndrome (34). Raised plasma hydroxybutyrylcarnitine and urinary 3-hydroxyglutarate are diagnostic of a rare type of congenital HH [*hydroxyacyl-Coenzyme A dehydrogenase (HADH)* deficiency] (35).

Some types of HH are elicited only after provocation testing. For example in patients who have the HI/HA syndrome and *HADH*, protein/leucine loading precipitates hypoglycaemia (36). Patients with exercise-induced HH will require a formal exercise test and or a pyruvate load to demonstrate post-exercise induced HH (37,38). In some patients, a positive glycaemic response (rise in the plasma glucose concentration of >1.5 mmol/L from baseline) following an intramuscular/intravenous injection of glucagon at the time of hypoglycaemia provides supportive evidence (39). A glycaemic response to a subcutaneous dose of octreotide may also aid diagnosis, along with decreased serum levels of insulin growth factor binding protein-1 (IGFBP-1) as insulin suppresses the transcription of the *IGFBP-1* gene (40).

Transient Forms of Hyperinsulinaemic Hypoglycaemia

There is no precise definition of transient HH, but if the hypoglycaemia resolves spontaneously within a few days (or up to a week) then it might be considered to be transient. Transient HH typically develops in newborns with certain risk factors [such as maternal diabetes mellitus, the use of intravenous dextrose given during labour, intrauterine growth restriction (IUGR), and perinatal asphyxia (Table 1)]. Some newborns with IUGR and asphyxia have a severe and protracted form of HH which requires treatment with diazoxide (41). The underlying molecular mechanisms in transient cases are not known, but some cases are due to mutations in *HNF4A* and *HNF1A* (33). In addition, transient HH has been described in some newborns with no underlying risk factors (42).

Genetic Forms of Hyperinsulinaemic Hypoglycaemia

The genetic basis of CHI involves defects in genes that encode key proteins involved in the regulation of insulin release from the pancreatic β-cell. These defects lead to disturbances in glucose-stimulated insulin secretion and inappropriate release of insulin from pancreatic β-cells. Currently, mutations in 12 genes have been reported to cause CHI and more recently there have been isolated case reports of potential novel genetic mechanisms in patients with CHI and other syndromic features. The underlying molecular mechanisms that causes CHI in the vast majority of patients who are diazoxide responsive are still unknown. Table 2 lists the transient and persistent causes of HH.

Table 1. Diagnostic criteria for hyperinsulinaemic hypoglycaemia

Cardinal diagnostic criteria	Low plasma glucose <3 mmol/L with;
	Detectable serum insulin
	Detectable C-peptide (superior to insulin, as is more stable in blood)
Biochemical features of insulin effects	Suppressed/low β- hydroxybutyrate and acetoacetate
	Suppressed/low serum free fatty acid
Clinical evidence of insulin effects	Increased requirement of glucose infusion rate (>8 mg/kg/min)
	Positive glycaemic (>1.5 mmol/L) response to intramuscular/intravenous glucagon
Supportive evidence (when diagnosis is in doubt or difficult)	Positive glycaemic response to a subcutaneous/intravenous dose of octreotide
	Low serum levels of IGFBP-1 (insulin negatively regulates the expression of IGFBP-1)
	Suppressed branch chain (leucine, isoleucine and valine) amino acids
	Provocation tests (leucine loading or exercise testing) may be needed in some patients
	Normal lactic acid
	Normal plasma hydroxybutyrylcarnitine*
	Normal ammonia**
	Appropriate counterregulatory hormone response***
	- Cortisol >20 mcg/dL (500 nmol/L)
	- Growth hormone >7 ng/mL

*Elevated in hyperinsulinaemic hypoglycaemia due to hydroxyacyl-CoA dehydrogenase gene mutation, **Elevated in hyperinsulinism-hyperammonemia (HI-HA) syndrome due to glutamate dehydrogenase 1 gene mutation, ***Counterregulatory hormone response may be blunted in spontaneous, particularly recurring hypoglycaemia, IGFBP-1: insulin-like growth factor binding protein-1

Genetics of Hyperinsulinaemic Hypoglycaemia

a) Pancreatic β-cell KATP Channel Defects

K_{ATP} channels are located in the β-cell membrane and transduce the metabolic signals generated by glucose metabolism to regulate insulin secretion (33). The K_{ATP} channel complex is composed of four outer, sulphonlyurea receptor 1 (SUR1) subunits that are encoded by the *ATP Binding Cassette Subfamily C Member 8 (ABCC8)* gene and the four inner, pore-making, Kir6.2 channel proteins, encoded by the *Potassium Voltage-Gated Channel Subfamily J Member 11 (KCNJ11)*. Both these genes are located on chrosome 11p15.1. The SUR1 component regulates the activity of the Kir6.2 proteins and functions as the binding site for the K_{ATP} channel opener (diazoxide) and sulphonylureas (43,44). The inner Kir6.2 protein forms a pore allowing potassium influx across the β-cell membrane. A change in the ratio of ATP to ADP causes closure of the K_{ATP} channel and triggers depolarisation of the cell membrane, activating the voltage-gated calcium channels (45). This in turn causes insulin release through exocytosis (46,47,48).

Mutations in the genes encoding K_{ATP} channel proteins are the most common cause of severe CHI (49,50). Recessive inactivating (or loss-of-function) K_{ATP} channel gene mutations predominantly cause medically unresponsive diffuse CHI (4,6,51,52). These mutations can either inhibit the trafficking of channel proteins (SUR1) to the plasma membrane or channel activity (33). Autosomal dominantly inherited mutations usually cause milder forms of CHI (53,54). Recently a novel phenomenon, describing the combination of heterozygous mutations in the *ABCC8* and *KCNJ11* genes has been described (55).

b) Glutamate Dehydrogenase (GDH) and Hyperinsulinaemia-hyperammonaemia Syndrome (HI/HA)

The *glutamate dehydrogenase 1 (GLUD1)* gene encodes for the mitochondrial matrix enzyme, GDH which catalyzes the oxidative deamination of glutamate to α-ketoglutarate and ammonia (56). GDH is allosterically activated by the amino acid leucine and inhibited by guanosine-5'-triphosphate (GTP) (57). *GLUD1* mutations decrease the sensitivity of the allosteric inhibitor, GTP, thereby resulting in a gain-of-function of the GDH enzyme. Dominantly inherited *GLUD1* mutations are associated with fasting and leucine (protein) induced postprandial HH, with elevated plasma ammonia (also known as HI/HA syndrome) concentration. Interestingly in a mutant GDH mouse model carrying the *H454Y* mutation, in addition to the loss of GTP inhibition on GDH activity, there was also inhibition of glucagon secretion (58). This inhibition of glucagon secretion may also contribute to the development symptomatic hypoglycemia in these patients (58).

GLUD1 mutations are the second most common cause of CHI. Studies to date have identified mutations in exons 6, 7, 11 and 12 and 13 (34,59). Although *GLUD1* activating mutations are mostly *de novo*, autosomal dominant forms have also been reported (59,60). HI/HA syndrome patients are diazoxide responsive and in some cases dietary protein restriction might be necessary. Patients with *GLUD1* mutations have been reported to develop epileptic seizures regardless of the severity and frequency of hypoglycaemic episodes. Urinary α-ketoglutarate is elevated in patients with HI/HA syndrome (61).

c) Mutations in Mitochondrial L-3-Hydroxyacyl-CoA Dehydrogenase (HADH) and CHI

HADH or short chain L-3-Hydroxyacyl-CoA dehydrogenase is another mitochondrial enzyme that is involved in the penultimate step of β-oxidation of fatty acids. This gene is most abundantly expressed in pacreatic islet cells, while also present in other extrapancreatic tissues such as the liver, kidneys, muscle and heart (62). The *HADH* gene has 8 exonic regions and autosomal recessive loss-of-function mutations impair the enzymatic inhibitory effect of HADH on GDH (63,64,65). This in turn causes a rise in incracellular ATP and inappropriate -leucine sensitive- HH. These observations suggest that GDH plays a pivotal role in fatty acid and amino acid metabolism to control insulin secretion (33). The serum ammonia level is normal in these patients. *HADH* gene mutations can lead either to severe neonatal HH or to mild, late (even adult) onset, protein-induced HH (66,67). Mutations of *HADH* gene have been reported as one of the most common cause of diazoxide responsive CHI in consanguineous pedigrees. Therefore, *HADH* sequence analysis is recommended for all patients with diazoxide-responsive HH when recessive inheritance is suspected (68). Patient with *HADH* mutations may have elevated plasma concentrations of 3-hydroxy-butyryl-carnitine and urinary 3-hydroxy-glutaric acid (35,65).

d) Activating Mutations in GCK and CHI

GCK catalyses glucose to glucose-6-phosphate conversion as substrate for the glycolytic pathway leading to ATP generation and glucose-dependent insulin release. *GCK* has high affinity for glucose, serving as a glucose-sensor in pancreatic β-cells. The *GCK* gene has 12 exons and encodes the enzyme, *GCK*. *GCK* can be found in the pancreatic β-cells, liver and brain (69). Dominant activating mutations in *GCK* cause alteration in both protein structure and function. The affinity of mutated *GCK* enzyme for glucose increases, thereby the threshold for glucose-stimulated insulin release is decreased (70,71). Patients with *GCK* mutations can have a wide range of clinical presentations. These vary from

Table 2. Transient and permanent causes of hyperinsulinaemic hypoglycaemia

Transient causes of HH
Maternal diabetes mellitus (gestational and insulin-dependent)
Intrauterine growth restriction
Perinatal asphyxia
Rhesus isoimmunisation
HNF4A, HNF1A mutations
Genetic causes of HH
Mutations in the genes encoding K_{ATP} channel proteins SUR1 and Kir6.2
ABCC8
KCNJ11
Mutation in the genes involved in the regulation of insulin secretion
GLUD1
HADH
GCK
SLC16A1
HNF1A
HNF4A
Recently described gene mutations
UCP2
HK1
PGM1
PMM2
FOXA2 (single case report)
CACNA1D (single case report)
Metabolic causes of HH
Congenital disorders of glycosylation (CGD type 1a, 1b and 1d)
Tyrosinaemia type 1
Other syndromes associated with HH
Beckwith-Wiedemann syndrome
Kabuki's syndrome
Trisomy 13
Central hypoventilation syndrome
Leprechaunism (insulin resistance syndrome)
Mosaic Turner syndrome
Sotos syndrome
Usher syndrome
Timothy syndrome
Costello syndrome
Miscellaneous causes of HH
Postprandial HH
Insulin gene receptor mutation
Dumping syndrome
Noninsulinoma pancreatogenous hypoglycaemia syndrome (adults)
Insulin autoimmune syndrome (mostly adults)
Bariatric surgery (adults)
Insulinoma
Non-islet cell tumour hypoglycaemia (adults)
Factitious hypoglycaemia
Drug-induced

HH: hyperinsulinaemic hypoglycaemia, *HNF4A: hepatocyte nuclear factor 4A, HNF1A: hepatocyte nuclear factor 1A,* K_{ATP}: adenosine triphosphate-sensitive potassium channels, SUR1: sulphonlyurea receptor 1, Kir6.2: inwardly rectifying potassium, *ABCC8: ATP binding cassette subfamily C member 8, KCNJ11: potassium voltage-gated channel subfamily J member 11, GLUD1: glutamate dehydrogenase 1, HADH: hydroxyacyl-CoA dehydrogenase, GCK: glucokinase, SLC16A1: solute carrier family 16 member 1, UCP2: uncoupling protein 2, HK1: hexokinase 1, PGM1: phosphoglucomutase 1, PMM2: phosphomannomutase 2, FOXA2: forkhead box protein A2*

severe, neonatal-onset HH which is medically unresponsive and requiring surgery to mild, adult-onset HH which may be asymptomatic (3,72,73,74,75).

e) Mutations in Solute Carrier Family 16 Member 1 (SLC16A1) and Exercise-induced CHI

Monocarboxylate transporter 1 (MCT1) protein, encoded by the *SLC16A1* gene, is involved in the transport of pyruvate and lactate across the β-cell membrane. These monocarboxylates (pyruvate and lactate) serve as substrates for the Krebs cycle. Under physiological conditions the *SLC16A1* gene is silenced in pancreatic β-cells suggesting that both pyruvate and lactate are prevented from stimulating insulin secretion (33). Dominant gain-of-function mutations in the promotor region of *SLC16A1* cause increased expression of MCT1 in β-cells. This in turn leads to glycolysis-generated pyruvate to continually enter the Kreb's cycle and stimulate insulin secretion in states of low plasma glucose during anaerobic exercise, and in particular strenuous exercise (76). A pyruvate load or excercise test may precipitate HH and may be used for diagnostic purposes (38). These patients are often diazoxide responsive and avoiding strenuous exercise is advised (37).

f) Hepatocyte Nuclear Factor (HNF) 1A&4A (HNF1A&4A) and CHI

The HNFs, HNF1–α and HNF4-α, are transcription factors for nuclear hormone receptors expressed in pancreatic β-cells and regulate glucose-dependent insulin secretion (77,78). The hepatocyte nuclear factors 1A and 4A genes (*HNF1A/HNF4A*) encode for the HNF1-α and HNF4-α proteins, respectively. Heterozygous loss-of-function mutations in *HNF4A* and *HNF1A* lead to HH in the newborn period and maturity onset-diabetes (type 1 and 3) later in life (79,80,81,82). CHI due to mutations in both *HNF1A* and *HNF4A* are characterized by macrosomic birth and mild transient to severe diazoxide-responsive HH (6,13,52,79,83,84,85). CHI due to *HNF4A* gene has been reported with increased levels of glycogen in erythrocytes, elevated liver transaminases and increased echogenicity on liver ultrasonography, suggesting a glycogenosis-like phenotype (86,87). In some patients with diazoxide responsive HH, mutations in *HNF1A* and *HNF4A* may be common (85,88).

g) Mutations in the Mitochondrial Uncoupling Protein 2 (UCP2) and CHI

UCP2, an inner mitochondrial carrier protein which encoded by the *UCP2* gene, is widely expressed in tissues, including pancreatic islets (89,90). *UCP2* mediates proton leak across the inner mitochondrial membrane, thereby inhibiting ATP generation through mitochondrial oxidative metabolism and negatively regulates glucose mediated insulin secretion (90,91). Inactivating heterozygous mutations of the *UCP2* gene would therefore, enhance glucose oxidation and increase intracellular ATP synthesis leading to HH (90,92). CHI due to *UCP2* mutations can present with a clinical phenotype ranging from transient HH to prolonged HH (28,90,93). In one study *UCP2* variants were found in 2.4% from a cohort of 211 diazoxide responsive patients (28). However, in a more recent study, no protein truncated variants were detected in the *UCP2* gene among 206 diazoxide responsive patients (94). The only variant detected was considered to be a common polymorphism. This suggests, therefore, that the role *UCP2* in CHI needs further investigation.

h) Somatic overexpression of Hexokinase 1 (HK1) and CHI

HK1 is located on chromosome 10 and encodes the enzyme; HK1. Hexokinases are a group of enzymes that catalyse the first step of glucose metabolism, of which HK1 is the predominant enzyme. It catalyses the phosphorylation of glucose to produce glucose-6-phosphate as substrate for glycolysis. Normally, *HK1* expression is silenced in the pancreatic β-cells. Recently however, a report identified a dominant gain-of-function mutation in the *HK1* gene in a family with "idiopathic hypoglycaemia of infancy" (17). Further evidence for the role of overexpression of HK1 has been reported in an *in vitro* study evaluating pancreatic specimens of five CHI cases which showed inappropriate expression of "HK1" in a subset of pancreatic β-cells. In these pancreatic specimens the K_{ATP} channel was functional but there was inappropriate insulin secretion at low plasma glucose levels (1 mmo/L) (95).

i) Phosphoglucomutase 1 (*PGM1*) Gene Mutations and CHI

PGM1 catalyses the reversible conversion of glucose-6-phosphate to glucose-1-phosphate involved in glycogen metabolism. Recently, a recessive loss-of-function mutation in the *PGM1* gene that encodes the enzyme *PGM1* has been shown to be associated with hypoglycaemia, similar to glycogenosis (18). Patients with these inactivating mutations have an exaggerated glucose-mediated insulin secretion and therefore present with fasting hyperketotic hypoglycaemia, as well as postprandial HH (15).

j) Phosphomannomutase 2 (*PMM2*) Gene Mutations and CHI

The enzyme *PMM2* is involved in glycosylation and the *PMM2* gene has recently been reported to cause HH as well as congenital polycystic kidney disease in 17 children from 11 unrelated families (16). The group reported a promoter mutation (c.-167G>T) in the *PMM2* gene in all affected patients. This mutation has been shown to alter insulin secretion from pancreatic β-cells.

k) Mutations in CACNA1D and CHI (Single Case Report)

CACNA1D encodes an L-type voltage-gated calcium channel that plays a pivotal role in the regulation of insulin secretion from pancreatic β-cells. A patient with a *CACNA1D* gene mutation has been reported with HH, heart defects and severe hypotonia (20) but the molecular mechanism leading to HH is still not clear.

l) Mutations in Forkhead Box Protein A2 (*FOXA2*) and CHI (Single Case Report)

A case has been reported of a mutation in *FOXA2* with hypopituitarism, HH and endoderm-derived organ abnormalities (19). Again the moleclar basis of the HH observed in the patient was not elucidated.

Hyperinsulinaemic Hypoglycaemia Management

The cornerstone of clinical management involves the early diagnosis and starting of appropriate therapy for patients with all forms of HH. The aim is to keep plasma glucose levels above 3.5 mmol/L given that the brain is deprived of alternative substrates. The treatment options includes medical, surgical or sometimes combination therapies.

Emergency Management

Parenteral glucose infusion: If the patient is unable to take an oral feed then 2 mls/kg of 10% glucose should be administered intravenously as a bolus. In some instances, a repeat bolus may be required, but further repeated boluses should be avoided, as the bolus of glucose is a potent trigger for insulin secretion. Normoglycemia should be achieved by delivering a continuous intravenous glucose infusion starting with 6-8 mg/kg/min. Patients with HH may require > 25 mg/kg/min of intravenous glucose infusion to maintain normoglycaemia.

Glucagon administration: Glucagon is a key counter-regulatory hormone and is used as a first line therapy for managing CHI patients, particularly in emergency situations where patients are unable to take oral feed and/or intravenous access is difficult to obtain (32,96). Glucagon, in the short-term, induces glycogenolysis, gluconeogenesis and lipolysis and causes a rapid increase in plasma glucose within a few minutes after administration. The recommended single dose is 0.5-1 mg via intramuscular or subcutaneous injection. Glucagon, in high doses (over 1 mg), can cause rebound hypoglycemia due to a paradoxical increase in insulin secretion (97). Long-term non-surgical management of CHI using continuous subcutaneous glucagon infusion at a rate of 5-10 mcg/kg/hour in combination with octreotide have been reported (98,99).

Frequent feeding: Frequent feeding with high calorie carbohydrate feeds may reduce the frequency and severity of hypoglycaemic episodes. However, patients wth CHI, particularly those on diazoxide therapy usually have food aversion. Therefore a percutaneous gastrostomy is sometimes recommended to allow frequent (or continuous) feeding (100,101). Using complex carbohydrate such as uncooked cornstarch may decrease the hypoglycaemic episodes and improve fasting tolerance during a prolonged overnight fast in children over the age of one year.

Long-term Management

A long-term management plan should be individualized for each patient and aim to normalize plasma glucose levels, provide an age-adjusted fasting tolerance and avoid neurological symptoms associated with hypoglycemia. Pharmacological therapy should be introduced one at a time to gauge the response and carefully monitored for side effects.

Diazoxide: Diazoxide, a K_{ATP} channel opener, is invaluable for managing many patients with CHI (1,32,96,102). Diazoxide is usually effective in all forms of CHI where the K_{ATP} channel function is intact but patients with recessive (and some dominant) K_{ATP} channel mutations do not respond to diazoxide (1). Diazoxide functions by binding to the SUR1 subunit of K_{ATP} channel. Thus, it requires a functionally intact K_{ATP} channel. Diazoxide responsiveness has been the key for molecular genetics analysis, differential diagnosis and management strategies of CHI. In diazoxide unresponsive CHI cases, urgent genetic analysis for *ABCC8*/*KCNJ11* and ^{18}F-DOPA-PET/CT scan are indicated to identify those patients who could have the focal form of CHI. In a recent study, diazoxide responsive patients with CHI who carry paternally inherited *ABCC8* or *KCNJ11* mutations have been reported and thus it was suggested that these patients should also undergo scanning with ^{18}F-DOPA PET/CT (103).

The initial dose of diazoxide is 5 mg/kg/day, in three divided doses which can be increased up to a maximum dose of 15-20 mg/kg/day (104). The citeria for diazoxide responsiveness include an age adjusted fasting tolerance, able to maintain normoglycaemia and have a normal feeding plan. The most severe side effect that limits and requires treatment withdrawal is fluid retention, cardiac failure and the associated electrolyte imbalance. Diazoxide induced pulmonary hypertension is another life-threatening side effect which requires treatment withdrawal and therefore the FDA has issued a drug safety communication warning (105,106,107,108). In the newborn period a thiazide diuretic (such as chlorothiazide 7-10 mg/kg/day in two divided doses), is usually administered with diazoxide to prevent fluid retention. Other side effects of diazoxide therapy are described in Table 3 (33,102,109).

Octreotide: Octreotide, is an eight amino acid, synthetic, long-acting somatostatin analogue that inhibits insulin

secretion by binding to somatostatin receptors 2 and 5 (SSTR2 and SSTR5) (110). Activation of SSTR5 decreases insulin gene promoter activity, inhibits calcium mobilization and acetylcholine activity (111). Somatostatin also inhibits the K_{ATP} channel which results in reduced insulin secretion (96). The recommended initial dose of octreotide is 5 µg/kg/day given by subcutaneous injections (or as a continuous infusion) at 6-8h intervals with a maximum dose of 30-35 µg/kg/day. Long-term, continuous, subcutaneous octreotide infusion with an insulin pump has also been reported as a feasible alternative to surgery for patients with monoallelic K_{ATP}-channel mutations (112). The first response to octreotide administration is usually hyperglycaemia followed by a blunted effect after 48 hours (tachyphylaxis). Thus dose adjustment may be required (32,113,114). Although various side effects have been reported in case reports, in a study evalutaing the long-term safety and efficacy of octreotide in a large series of CHI patients, it was found to be a safe and effective treatment for diazoxide unresponsive CHI patients (102,115,116,117,118,119,120,121,122,123) (Table 3). The effect of octreotide on linear growth have been found clinically insignificant (102,117,123). In a recent clinical trial, monitoring the serum concentration of octreotide is recommended for dose titration, in order to avoid paradoxically diminished effectiveness and to reduce the side effects, thereby achieving optimal doses for highest efficacy and safety (123).

Long-acting somatostatin analogs: As conventional octreotide therapy requires multidose daily injections, this causes a burden to the patients and family, reduces adherence to the treatment and impacts negatively on quality of life (QoL). Monthly injection of long-acting somatostatin analogs have been described as an effective option in the management of CHI. Long-acting octreotide release (LAR) is formulated with biodegradable microspheres (124). This formulation increases the half-life with the advantage of being administrated every 28 days. Lanreotide is also a synthetic octapeptide and it can be adminstered every 28 days. LAR-octreotide and lanreotide have been used successfully in children with CHI, even in early infancy (102,125,126,127,128,129,130,131). Using LAR once every four weeks increases the treatment adherence and improves QoL (125).

Nifedipine: As the voltage gated calcium channel plays a key role in insulin secretion from the pancreatic β-cell, nifedipine, an L-type calcium channel blocker, has been used in the treatment of CHI (132). There have been several case reports demonstrating the effectiveness of Nifedipine in CHI patients. (133,134,135,136,137,138). In a recent study exclusively investigating long-term use of nifedipine in eleven CHI cases with *ABCC8* mutations, none of patients showed any improvement in glycemic control and patients continued to have hypoglycemic episodes (139). This suggests that mutations in the K_{ATP} channel genes might render the L-type calcium channel ineffective to therapy with nifedipine (139) The recommended dose is 0.25-2.5 mg/kg/day divided into 2-3 doses (96). Hypotension is an uncommon side-effect (96), especially at doses above 0.5 mg/kg/day (134) (Table 3).

New and Potential Future Therapies

Although our knowledge of the molecular basis of CHI has advanced, there are still challenges in managing patients who are diazoxide unresponsive. Most patients with diffuse CHI who are diazoxide unresponsove will typically require a near total pancreatectomy. In some patients, despite this major surgery, hypoglycemia persisted. Thus novel medical treatments are required to try and avoid a near total pancreatectomy which is not always curative.

Sirolimus: Sirolimus, an immunosuppressive agent with an anti-proliferative ability, inhibits the mammalian target of rapamycin (mTOR), a serine/threonine kinase (140). mTOR regulates cellular growth by stimulating protein synthesis and increasing mRNA translation initiation (141,142). The mechanism of action for mTOR inhibitors in CHI has not been fully elucidated. However, it is reported that there is constitutive activation and overexpression of p-mTOR on the plasmalemmal aspect of the acinar cells and activation on the plasmalemmal aspect of the ductal cells in the diffuse variant of CHI (143). Recently, another mechanism has been proposed; that sirolimus causes depletion of intracellular Ca^{2+} stores and alters mitochondrial activity, eventually leading to decreased insulin release (140). Upregulation of mTOR leads to increased insulin release from the pancreatic β-cells (144). Conversely, mTOR inhibition with rapamycin reduces insulin secretion as well as β-cell growth (145). Sirolimus can also enhance β-cell apoptosis and insulin resistance by reducing islet mass, insulin content and insulin sensitivity (140). mTOR also inhibits peroxisome proliferators-activated receptor-γ activity thereby affecting ketone body synthesis (146).

Sirolimus has been reported to be an effective and safe drug for severe, diazoxide unresponsive, diffuse CHI with no major side effects (147). Following the first report, significant numbers of cases have been reported (148,149,150,151,152,153,154). As sirolimus has potentially adverse effects (perhaps related to dose) arising from its immunosuppressive effects, measurement of the blood levels is vitally important for reaching an optimal therapeutic level. The most commonly reported adverse effects are stomatitis, increased risk of infection, immunosuppression,

Table 3. Drugs for medical therapy of hyperinsulinaemic hypoglycaemia

Route		Dose	Mode of action	Side effects
Conventional medicines				
Diazoxide	Oral	5-20 mg/kg/day, in 3 divided doses	Bind to SUR1 subunit of KATP channels, opens the channels and inhibits insulin secretion	**Common:** Water and salt retention, hypertrichosis, loss of appetite
			Needs an intact K_{ATP} channel to work properly	**Rare:** Cardiac failure, pulmonary hypertension, hyperuricaemia, blood dyscrasias (bone marrow suppression, anaemia, eosinophilia etc.), paradoxical hypoglycaemia
Chlorothiazide	Oral	7-10 mg/kg/day, in 2 divided doses	Prevents fluid retention, synergistic effects with diazoxide on KATP channels to inhibit insulin secretion	Hyponatraemia, hypokalaemia
Nifedipine	Oral	0.25-2.5 mg/kg/day, in 2-3 divided doses	Inhibits Ca-channels of the β-cell membrane	Hypotension
Octreotide	s.c	5-35 µg/kg/day, divided to 3-4 doses or continuous subcutaneous infusion	Activation of SSTR2 and SSTR5 inhibits calcium mobilization and acetylcholine activity, decreases insulin gene promoter activity, reduces insulin biosynthesis and insulin secretion	**Acute:** Anorexia, nausea, abdominal discomfort, diarrhoea, drug induced hepatitis, elevated liver enzymes, long QT syndrome, tachyphylaxis, necrotizing enterocolitis
				Long-term: Decreases intestinal motility, bile sludge and gallstone, suppression of pituitary hormones (Growth hormone, TSH)
Glucagon	s.c/i.m bolus or s.c/i.v infusion	0.02 mg/kg/dose or 5-10 µg/kg/hour infusion	G-protein coupled activation of adenylate cyclase, increases cAMP. Induces glycogenolysis and gluconeogenesis	Nausea, vomiting, skin rash and rebound hypoglycaemia in high doses (> 20 µg/kg/hour) due to paradoxical activation of insulin secretion
New medicines				
Rapamycin (sirolimus, everolimus)	Oral	An initial dose of 1 mg/m^2 per day may require dose adjustment according to blood sirolimus concentration aiming to keep between 5-15 ng/mL	mTOR inhibitor. Inhibit insulin release and β-cell proliferation through different mechanisms which have not been clarified yet	Immune suppression, mucositis, hyperlipidemia, elevation of liver enzymes, thrombocytosis, impaired immune response to BCG vaccine
Octreotide LAR/ Lanreotide	deep s.c	Dose is calculated using cumulative current multi-injection dose of octreotide and given as a single dose every 4 weeks or a total dose of 15-60 mg/every 4 weeks	These long acting somatostatin analogues have similar effects to daily multidose octreotide	Similar to daily multiple injection octreotide. However, long-term follow up is not known yet

SUR1: sulphonlyurea receptor 1, $K_{ATP:}$ adenosine triphosphate-sensitive potassium channels, s.c: subcutaneous, i.m: intramuscular, i.v: intravenous, SSTR2: somatostatin receptors 2, SSTR5: somatostatin receptors 5, TSH: thyroid-stimulating hormone, BCG: Bacillus Calmette-Guérin, mTOR: mammalian target of rapamycin, LAR: long-acting release

renal dysfunction, fatigue, pneumonitis and increased serum aminotransferase or lipid levels (155).

In a recent report evaluating the efficacy of sirolimus in 10 patients with diazoxide unresponsive CHI, mTOR inhibition has shown to be effective in only three patients (30%) with certain side effects (156). In addition, pancreatic tissue from two patients who did not respond to sirolimus showed no reduction in β-cell proliferation. Therefore it was claimed that inhibition of mTOR signaling does not down-regulate the β-cell proliferation in patients with CHI (156). Thus furthur studies, ideally in the form of clinical trails are required to assess the efficacy of mTOR inhibitors in CHI patients.

Glucagon-like peptide-1 Receptor Antagonist: Exendin-(9-39)

GLP-1 is an incretin hormone produced in enteroendocrine L-cells of the intestine in response to ingested nutrients (157). GLP-1 enhances insulin secretion by binding to a guanine nucleotide binding protein-coupled receptor (158), resulting in the activation of adenylate cyclase and generation of cAMP (159). GLP-1 stimulates insulin secretion by both protein kinase A-dependent and -independent mechanisms (160) and also inhibits glucagon secretion, hepatic glucose production, gastric emptying and appetite.

Exendin-(9-39) is a specific GLP-1 receptor antagonist in mice and humans (161,162). In *Sur-1* knock-out mice it was shown that Exendin-(9-39) decreases cAMP levels and inhibits insulin secretion thereby raising fasting plasma glucose levels (163). Another study demonstrated that exendin-(9-39) prevents hypoglycemia and maintains normoglycemia during a prolonged fast in individuals with K_{ATP} mutations (164). These promising results point to the GLP-1 receptor as a therapeutic target for K_{ATP} mutations. More recently, in the first population pharmacokinetic model of exendin-(9-39) in patients with CHI, the maximum recommended starting dose was determined to be 27 mg/kg/day, intravenously (165). This result informs the optimal dosing regimen for future clinical trials in neonates with CHI.

Ketogenic diet: CHI typically deprives the brain of both its main and alternative energy sources, being glucose and ketone bodies respectively. During the suckling period, ketone bodies constitute the main energy substrate for the brain. However, in the adult brain glucose is the main energy source (166). An increase in the ketone body concentration increases their oxidation rate in the brain (167,168). Thus, ketogenic diets have been used as an adjunctive therapeutic option in refractory epilepsy and in experimental models of ischemia and excitotoxicity (169). HH induces severe neuroglycopenia and also inhibits gluconeogenesis, glycogenolysis, lipolysis and, eventually, fatty acid oxidation which results in suppressed ketone body synthesis. This makes the brain more vulnerable to the neurological insult of hypoglycaemia. Maiorana et al (170) reported a trial ketogenic diet administered to a child with CHI due to a spontaneous *GCK* activating mutation and recurring hypoglycaemic episodes, despite medical therapy. After the first six months, the patient was free of epileptic seizures, with normalization of EEG and showed a marked recovery in psychological development and QoL (170). Although this treatment requires further investigation these initial findings suggest that a ketogenic diet could have a neuroprotective effect in selected cases of CHI.

Histologic Subtypes of Congenital Hyperinsulinaemic Hypoglycaemia

In terms of histology, there are three forms of CHI; focal, diffuse, and atypical disease (Figure 2). In focal CHI the

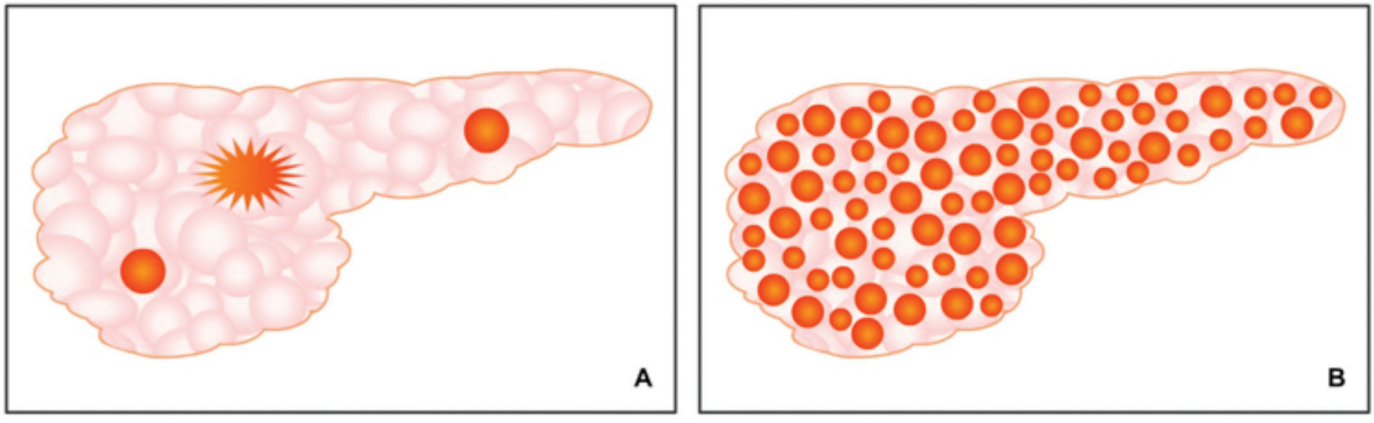

Figure 2. A schematic representation of focal and diffuse congenital hyperinsulinism. In the focal disease (A), the β-cell hyperplasia is limited to a certain are of the pancreas gland with a superficial or deep localization or invades as a tentacle shape. In the diffuse disease (B) there is a global β-cell hyperplasia throughout the whole panreas

abnormal pancreatic β-cells are localised to a specific region of the pancreas. Focal pancreatic lesions are generally 2-10 mm in size and appear as small regions of islet adenomatosis (nodular hyperplasia of islet-like cell clusters, including ductuloinsular complexes, Figure 3) (33). Islet cells in the lesion have large cytoplasm with dispersed abnormal nuclei of irregular shape (171).

Focal disease is mostly sporadic and is associated with a paternally inherited K_{ATP} channel mutations and the loss of maternal heterozygosity for 11p in the focal area (172). This in turn induces the expression of insulin-like growth factor 2, inhibits the tumor suppressor genes H19 and cyclin-dependent kinase inhibitor 1C and leads to β-cell proliferation (173). ^{18}F-DOPA-PET scanning is currently the only diagnostic imaging tool to accurately localize focal lesions (174). Pancreatic islets are able to uptake L-DOPA and convert it to dopamine through DOPA decarboxylase. The uptake of the positron emitting tracer ^{18}F-DOPA-PET is increased in β-cells with a high rate of insulin synthesis and secretion compared to unaffected areas (Figure 3). The sensitivity for detecting focal lesions varies between 88 and 94 % with an accuracy of 100 % (175). In a recent study ^{18}F-DOPA-PET/CT was found to be superior in localizing focal lesions compared to imaging with ^{68}Ga-DOTANOC PET/CT (176). Patients with focal CHI are usually unressponsive to medical therapy and require a surgical lesionectomy.

Diffuse disease accounts for about 60 % of all CHI cases and affects all the β-cells of the pancreas. Morphology of the islets of Langerhans typically show the presence of β-cells with abnormally large nuclei (Figure 3) (177). Patients with diffuse CHI mostly have either a homozygous recessive or a compound heterozygous mutations in K_{ATP} channel genes (8). Patients are usually unresponsive to medical therapy and require a near-total pancreatectomy (95-98 % removal).

In some cases pancreatic histology does not fit the typical focal or diffuse appearance and therefore atypical forms of

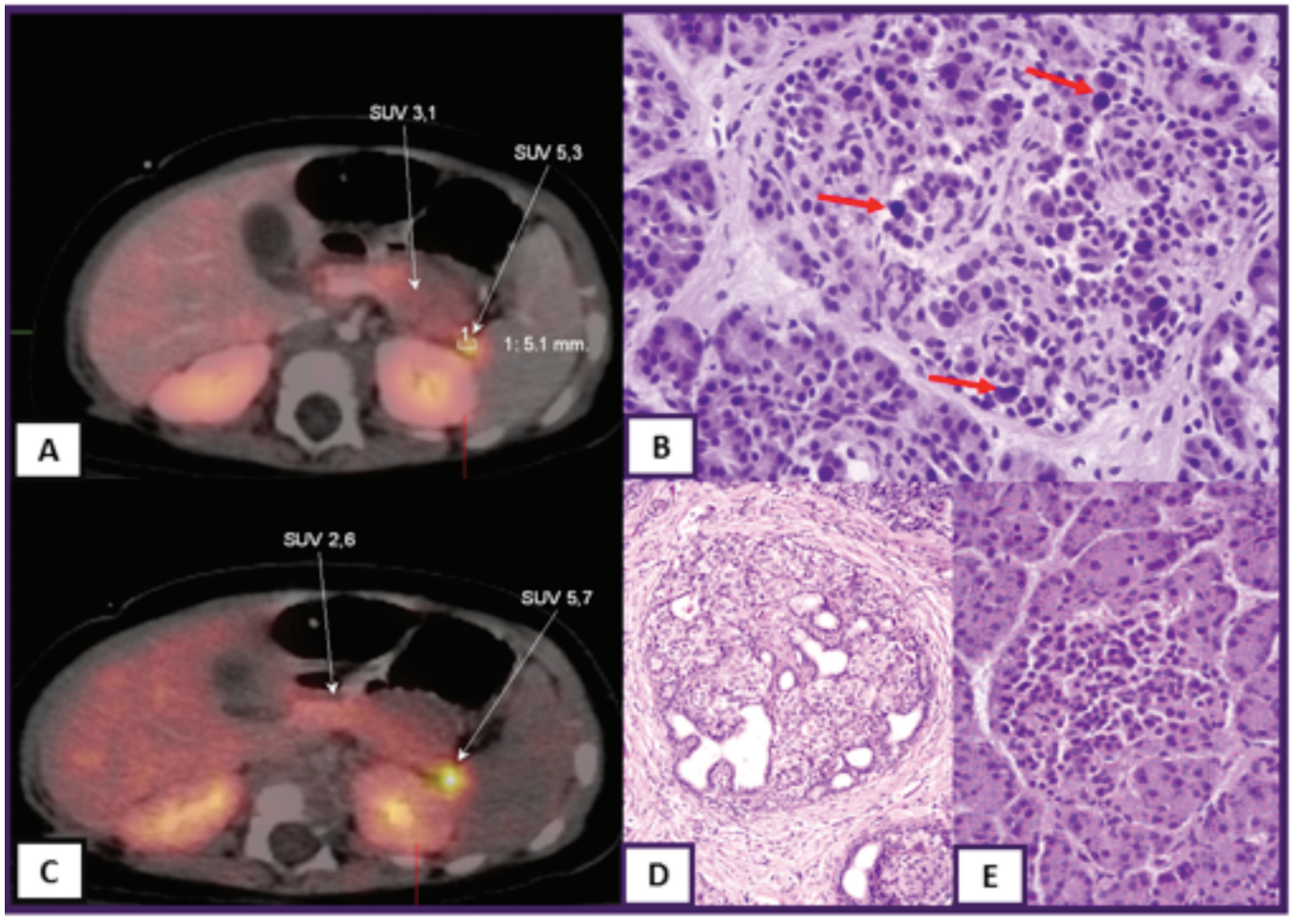

Figure 3. ^{18}F-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA)-positron emission tomography/computed tomography scan images of focal congenital hyperinsulinism (A and C), histological figure of diffuse (B) and focal (D) disease and normal pancreas islet cell (E). Standardized uptake value (SUV) 5.3 and SUV 5.7 indicate focal uptake of ^{18}F-DOPA, red arrows show large nuclei of β-cell in diffuse disease

CHI have been described (178,179,180). In atypical forms some islets show signs of hyperplasia interpersed with histologically normal looking islets. Some patients with CHI have morphological mosaicism including coexistence of two types of islet; large islets with cytoplasm-rich cells and occasional enlarged nuclei and shrunken islets with cells exhibiting little cytoplasm and small nuclei (173).

Surgical Therapy

Differentiation of the histological subtypes is essential for successful surgical outcome. Recent advances in the molecular genetics of CHI and imaging with ^{18}F-DOPA-PET/CT have changed the management of patients, particularly those with focal disease (177). In diffuse disease there is uptake of ^{18}F-DOPA throughout the pancreas on the PET/CT scan whereas in focal forms there is limited uptake of ^{18}F-DOPA in a localised region of the pancreas. Once this focal lesion is localised on the PET/CT it is possible to surgically remove the lesion and cure the patient of hypoglycemia (Figure 3) (181,182). Intraoperative frozen sections are important as these can both confirm the histological diagnosis and to determine the margin of resection (183).

Surgery for diffuse and atypical disease: Patients with diffuse and atypical disease usually require extensive surgery (subtotal- or near-total pancreatectomy). This procedure caries a high risk of developing pancreatic exocrine insufficiency and diabetes which requires life-long pancreatic enzyme replacement and insulin therapy (7,184,185,186,187). In near-total pancreatectomy, the tail, body, uncinate process and part of the pancreatic head are resected, leaving a rim of pancreatic tissue surrounding the common bile duct and along the duodenum (7). However, despite extensive resection (95-98 % of pancreatic tissue) some children continue to have HH (185). Diabetes can develop immediately after surgery or later during follow-up (184). Therefore, patients who undergo surgical resection should be monitored for glucose metabolism and diabetes (184,185,186,187).

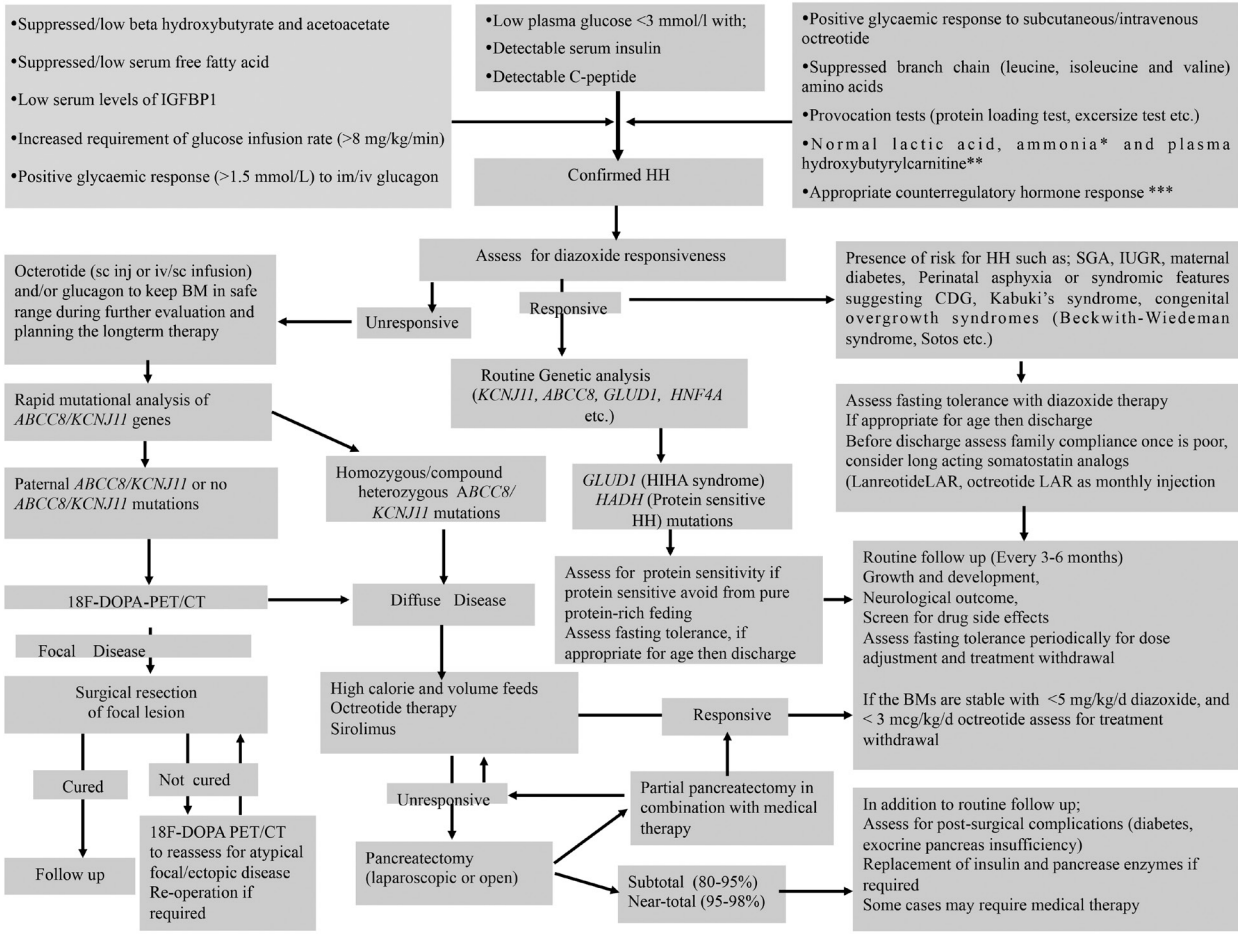

Figure 4. An algorithm for the diagnosis and management of hyperinsulinaemic hypoglycaemia

HH: hyperinsulinaemic hypoglycaemia, IGFBP-1: insulin growth factor binding protein-1, *HNF4A: hepatocyte nuclear factor 4A, ABCC8: ATP binding cassette subfamily C member 8, KCNJ11: potassium voltage-gated channel subfamily J member 11, GLUD1: glutamate dehydrogenase 1, HADH: hydroxyacyl-CoA dehydrogenase, LAR: long-acting release, IUGR: intrauterine growth restriction, CDG: congenital disorders of glycosylation, SGA: small for gestational age*, ^{18}F-DOPA-PET/CT: ^{18}F-fluoro-L-dihydroxyphenylalanine-positron emission tomography/computed tomography

Follow up and Outcome of Congenital Hyperinsulinism

The management of patients with severe CHI is challenging and requires a multi-disciplinary team approach which should include clinicians, surgeons, specialized pathologists, geneticists, nurse specialists and dietitians. In studies evaluating the long-term outcome of patients with HH, a high frequency of neurodevelopmental retardation and various neurological disorders, including epilepsy and microcephaly, have been reported (187,188,189). Severity of the disease (based on maximal diazoxide dose) and early presentation (< 7 days following birth) were associated with abnormal neurodevelopment, while gender, underlying genetic etiology or the histopathological form of HH were not related to the neurological outcome (189). In a recent study evaluating long-term neurodevelopmental outcome of 60 patients with CHI, just under two fifths of cases were shown to be affected with motor deficits (38.6%) followed by speech problems (26.9%), cognitive deficits (15.8%) and social-emotional problems (9.4%), with no correlation between outcome and genetic background (190). Therefore, neurological development should be closely followed up, regardless of the underlying etiology and histopathological type.

Figure 4 outlines management and follow-up of patients with congenital HH.

Conclusions and Future Directions

CHI is the most common cause of severe hypoglycaemia in the newborn and childhood period. The molecular basis of CHI involves defects in key genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF1A, HNF4A, UCP2, HK1, PGM1, PMM2 and FOXA2*) which regulate insulin secretion. Rapid genetic analysis, imaging with ^{18}F-DOPA-PET/CT scan, potential new medical therapies and development in surgical techniques have improved the management and outcome of the disease. Further research is needed to identify the underlying molecular basis of CHI, especially in patients who are diazoxide responsve. Novel, routinely available imaging techniques should be developed so that patients all over the world can have access to these facilities.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Hüseyin Demirbilek, Khalid Hussain, Design: Hüseyin Demirbilek, Khalid Hussain, Data Collection or Processing: Hüseyin Demirbilek, Khalid Hussain, Analysis or Interpretation: Hüseyin Demirbilek, Khalid Hussain, Literature Research: Hüseyin Demirbilek, Khalid Hussain, Writing: Hüseyin Demirbilek, Khalid Hussain.

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REVIEW

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Genetic Causes of Rickets

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Abstract

Rickets is a metabolic bone disease that develops as a result of inadequate mineralization of growing bone due to disruption of calcium, phosphorus and/or vitamin D metabolism. Nutritional rickets remains a significant child health problem in developing countries. In addition, several rare genetic causes of rickets have also been described, which can be divided into two groups. The first group consists of genetic disorders of vitamin D biosynthesis and action, such as vitamin D-dependent rickets type 1A (VDDR1A), vitamin D-dependent rickets type 1B (VDDR1B), vitamin D-dependent rickets type 2A (VDDR2A), and vitamin D-dependent rickets type 2B (VDDR2B). The second group involves genetic disorders of excessive renal phosphate loss (hereditary hypophosphatemic rickets) due to impairment in renal tubular phosphate reabsorption as a result of FGF23-related or FGF23-independent causes. In this review, we focus on clinical, laboratory and genetic characteristics of various types of hereditary rickets as well as differential diagnosis and treatment approaches.
Keywords: Rickets, hereditary, genetic, vitamin D dependent, hypophosphatemic rickets

Introduction

Rickets is a disease of growing bone seen in children and adolescents due to deficiency in calcium, phosphate and/or vitamin D, leading to inadequate mineralization of osteoid tissue in the growth plate and bone matrix (1). The most frequent cause of rickets in Turkey, as well as in the rest of the world, continues to be nutritional vitamin D deficiency (1,2). Genetic causes of rickets (hereditary rickets) are rare: accounting for about 13% of total rickets (3).

They can be divided into two groups: vitamin D-dependent rickets which is caused by mutations either in enzymes involved in the vitamin D biosynthesis or vitamin D receptor (4), and hypophosphatemic rickets (HR) which is caused by impaired renal tubular phosphate reabsorption or transport due to genetic disorders associated with phosphatonins or phosphate co-transporters (5).

Calcium is one of the most common minerals in the body and it is mainly derived from dietary sources (6). It is essential for bone metabolism and various biological functions (6). While more than 99% of total calcium is stored in bone tissue as calcium-phosphate complex, less than <1% is distributed between intracellular and extracellular compartments (7). Of the <1% calcium outside bone tissue, 40% is bound to proteins, 9% is contained in ionic complexes and the remaining 51% is in the form of free Ca^{2+} ions that are the biologically active portion of body calcium (6,8). The ionized calcium balances the calcium pool in the intracellular-extracellular space and plays an important role in bone metabolism. This balance is achieved through the collective action of several hormones such as parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25(OH)2D] and organs such as the kidney, bone and intestinal system (7,8). If serum calcium levels decrease, calcium-sensing receptors located on parathyroid cells mediate increased secretion of PTH, which binds to PTH 1 receptor (PTH1R, expressed in high levels in bone and kidney) to promote calcium resorption from bone and reabsorption from kidneys. PTH also activates 25-hydroxyvitamin D3-1α-hydroxylase, leading to increased 1,25(OH)2D synthesis, which promotes calcium absorption from intestines and reabsorption from proximal tubules of kidney (6,7,8).

Phosphorus is the most common anion in the human body. It is found in the form of inorganic phosphate and plays an important role in many biological processes such

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as bone mineralization, cell membrane integrity, nucleic acid and energy metabolism, signal transduction through phosphorylation of proteins and oxygen transport (9). In the adult male human, total body phosphorus is between 15 mol and 20 mol (12.0 g/kg), 80-90% of which is present in bone in the form of hydroxyapatite and the remaining 10-20% in soft tissue and extracellular spaces (9). Approximately two-thirds of dietary phosphate is absorbed via the sodium-dependent phosphate transporter 2B (NaPi-2b, encoded by the *SLC34A2* gene), the major transporter that mediates phosphate reabsorption in the small intestine, predominantly in the jejunum. The expression of NaPi-2b is regulated by 1,25(OH)2D, which induces transcriptional up-regulation of NaPi-2b in the small intestine and low phosphate can activate 1α-hydroxylase in the kidney (10). Phosphate in the circulation can be taken up into cells for various biological activities or can be stored in the bone tissue. Approximately 85% of phosphate is reabsorbed by the sodium-dependent phosphate transporter 2A (NaPi-2a, encoded by the gene *SLC34A1*) and the sodium-dependent phosphate transporter 2C (NaPi-2c, encoded by the gene *SLC34A3*) both of which are expressed in the proximal tubules of the kidney (5,11). 1,25(OH)2D increases intestinal absorption of phosphate and tubular reabsorption, whereas PTH decreases tubular reabsorption of phosphate (TRP). In addition, other molecules that have phosphaturic effects, so-called phosphatonins, have significant impact on the balance of serum phosphate by reducing TRP (12,13).

Vitamin D is a group of biologically inactive, fat-soluble prohormones that exist in two major forms: ergocalciferol (vitamin D2) produced by plants in response to ultraviolet irradiation and cholecalciferol (vitamin D3) derived from animal tissues or 7-dehydrocholesterol in human skin by the action of ultraviolet rays present in sunlight with a wavelength of 270-290 nm (4). The main source of vitamin D is endogenous synthesis. Normally only 0.04% of 25-hydroxyvitamin D [25(OH)D] and 0.4% of 1,25(OH)2D are free in plasma, the remainder being tightly bound to either a vitamin D transporter protein (85-88%; high affinity) or albumin (12-15%; low affinity) (14). Both forms need two-step hydroxylation for activation. The first step occurs in the liver where vitamin D is hydroxylated to the minimally active 25(OH)D by hepatic 25-hydroxlase. The second step occurs mainly in the kidney where 25(OH)D is further hydroxylated by 1α-hydroxylase to become the biologically active hormone 1,25(OH)2D (calcitriol), which binds to its nuclear receptor vitamin D responsive (VDR) to regulate gene transcription through heterodimerization with one of three retinoid X receptor (RXR) isoforms (RXRα, RXRβ, RXRγ) and binds to cognate VDR elements (VDREs) in the promoter region of target genes (14,15). The renal synthesis of 1,25(OH)2D is stimulated by PTH and suppressed by calcium, phosphate and 1,25(OH)2D itself with renal 1α-hydroxylase being stimulated by PTH, hypophosphatemia or hypocalcaemia. Alternatively, 25(OH)D and 1,25(OH)2D may be catabolized to 24,25(OH)D and 1,24,25(OH)2D, respectively, through 24-hydroxylation by 25-hydroxyvitamin D 24-hydroxylase to maintain calcium homeostasis (4,14).

1. Vitamin D-Dependent Rickets

Disorders in the biosynthesis of vitamin D or its receptor activity result in vitamin D deficiency [vitamin D dependent rickets, type 1A (VDDR1A) and type 1B (VDDR1B)] or resistance [type 2A (VDDR2A) and type 2B (VDDR2B)]. All of them present similar clinical and biochemical manifestations of rickets such as findings related to hypocalcemia (irritability, fatigue, muscle cramps, seizures) and rickets (craniotabes, delayed closure of fontanelles, frontal bossing, enlarged wrists, bowed legs, short stature, and bone pain) (Table 1) (1,4).

1.1. Vitamin D-Dependent Rickets Type 1A

This disease, also called hereditary pseudo-vitamin D deficiency, was first described by Prader et al in 1961 as an autosomal recessive, persistent infantile rickets that responded to high dose vitamin D (16). Fraser et al (17) later reported that this condition was caused by lack of the 1-alpha hydroxylase enzyme. It is now defined as VDDR1A, (MIM 264700). VDDR1A occurs as a result of mutations in the *CYP27B1* (cytochrome P450, family 27, subfamily B, polypeptide 1, MIM 609506) that encodes the 1-alpha hydroxylase enzyme (17,18). As a result, 25(OH)D cannot be converted to active 1,25(OH)2D, leading to clinical findings of rickets and vitamin D deficiency. To date, over 100 patients with 72 different mutations have been described in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php, accessed Nov 13, 2017) (4,14,19,20,21). Strikingly, in a genetically isolated population of French-Canadians in Quebec, the disease is found with the highest global incidence (1/2700) (4). The most commonly reported mutation in this region is 958delG, the "Charlevoix mutation".

There is some genotype-phenotype correlation: milder phenotype is usually associated with mutations with residual enzyme activities (*E189G*, *G102E* and *L343F*) (22,23,24,25). Some milder cases may be missed and thus VDDR1A might be more common than is reported.

The disease is clinically similar to the phenotype of nutritional vitamin D-deficient rickets. The cases are usually normal at birth. However, growth retardation, skeletal

Table 1. Laboratory characteristics of rickets associated with vitamin D metabolism

Disease	Inheritance	Genetic defect	Protein	Serum calcium	Serum phosphate	ALP	PTH	25(OH)D	1,25(OH)2D	Urinary calcium/ creatinine
Calcium deficiency rickets	-	-	-	↓ or N	N or ↓	↑	↑	N	↑ or N	↓
Nutritional vitamin D deficiency	-	-	-	↓ or N	N or ↓	↑	↑	↓	↓ or N or ↑	↓
Vitamin D dependent rickets Type 1A (VDDR1A)	Autosomal recessive	*CYP27B1* mutation	1-alpha hydroxylase	↓ or N	N or ↓	↑	↑	N or ↑	↓ or N	↓
Vitamin D dependent rickets Type 1B (VDDR1B)	Autosomal recessive	*CYP2R1* mutation	25-hydroxylase	↓ or N	N or ↓	↑	↑	↓	N or ↓	↓
Vitamin D dependent rickets Type 2A (VDDR2A)	Autosomal recessive	*VDR* mutation	Vitamin D receptor	↓ or N	N or ↓	↑	↑	N	↑	↓
Vitamin D dependent rickets Type 2B (VDDR2B)	unknown	*HNRNPC* overexpression	Heterogeneous nuclear ribonucleoprotein C	↓ or N	N or ↓	↑	↑	N	↑	↓

ALP: alkaline phosphatase, PTH: parathyroid hormone, N: normal, VDR: vitamin D responsive, HNRPC: heterogeneous nuclear ribonucleoprotein C, 1,25(OH)2D: 1,25-dihydroxyvitamin D, 25(OH)D: 25-hydroxyvitamin D

deformities, muscle weakness, bone pain, muscle spasms and hypocalcemic convulsions may occur in the first year of life. The first observed findings in bone and joints include deformities such as craniotabes, metaphyseal enlargement, prominence of costochondral joints (rachitic rosary), delayed closure of the anterior fontanel, Harrison's grooves and thoracic anomalies (1,26).

Similar to cases of nutritional rickets, typical cases with VDDR1A present with hypocalcemia, hypophosphatemia and increased serum levels of alkaline phosphatase (ALP) and PTH (Table 1). In contrast to nutritional rickets, levels of 25(OH)D are generally normal and 1,25(OH)2D are low (20). Some patients may be misdiagnosed as nutritional rickets and thus incorrectly treated with high dose vitamin D, leading to very high levels of 25(OH)D. Renal calcium excretion is low in these patients. In addition, hyperchloremic metabolic acidosis and hyperaminoaciduria secondary to PTH elevation can occur (4). Inappropriately normal 1,25(OH)2D levels in the presence of hypocalcemia can also be found in some patients with VDDR1A (20,27). Some cases might also be normocalcemic and a misdiagnosis of HR might be made before the detection of significantly elevated PTH levels (20).

Proper treatment of the disease includes administration of calcitriol, 1,25-dihydroxyvitamin D3 or alfacalcidol, 1 alpha-hydroxy-vitamin D3 in physiological doses (10-20 ng/kg/day, 2 doses), which will gradually improve clinical, biochemical and radiological findings (26). In addition, it is recommended to add 50-75 mg/kg/day of elemental calcium at the beginning of treatment. On follow-up, effective management should result in low-normal serum levels of calcium (8.5-9 mg/dL), normal phosphate levels and high-normal PTH values (4,26). High-normal levels of serum calcium might lead to hypercalciuria and subsequent development of nephrocalcinosis. Regular monitoring of 24-hour urinary calcium excretion and keeping the urine calcium excretion below 4 mg/kg/day is recommended (4,5,26). The degree of calciuria can also be assessed with spot urine calcium/creatinine ratios, for which varying normal ranges exist for different age groups: < 0.8 mg/mg (≤6 months of age), < 0.6 mg/mg (7-12 months), < 0.53 mg/mg (1-3 years), < 0.39 mg/mg (3-5 years), < 0.28 mg/mg (5-7 years) and < 0.21 mg/mg (> 7 years) (28).

1.2. Vitamin D Dependent Rickets Type 1B

VDDR1B (MIM 600081) is an extremely rare autosomal recessive disorder, due to 25-hydroxylase deficiency. This disease was first described in 1994 by Casella et al (29) in two Nigerian siblings of two and seven years old. Skeletal deformities compatible with rickets, hypocalcemia,

hypophosphatemia, markedly elevated ALP and PTH, normal 1,25(OH)2D and low 25(OH)D levels were present. These siblings were diagnosed with 25-hydroxylase deficiency and showed clinical and laboratory improvement after high-dose vitamin D2 treatment. The gene encoding 25-hydroxylase (*CYP2R1*, MIM 608713) was described by Cheng et al (30) in 2003 and a homozygous *CYP2R1* mutation (*L99P*) was identified in one of the first reported Nigerian siblings (31). Currently, only four *CYP2R1* mutations are listed in the HGMD (accessed Nov 13, 2017). Apart from CYP2R1, there are five other cytochrome P450 enzymes (CYP27A1, CYP2J2/3, CYP3A4, CYP2D25 and CYP2C11) capable of catalyzing the initial 25-hydroxylation step (32). Indeed, a 20-month-old male patient has been described recently having hypocalcemic convulsions and rickets (33). His mother, maternal grandmother and aunt also have a history of hypercalcemic convulsion and skeletal deformities related with rickets in childhood. In all cases, hypocalcemia, hypophosphatemia, decreased 25(OH)D, markedly elevated ALP and PTH are present. Interestingly, a *CYP2R1* mutation has not been found in this kin, suggesting that another gene may be involved in 25-hydroxylation. Calcitriol is the only choice of treatment for the disease (10-20 ng/kg/day, 2 doses).

1.3. Vitamin D Dependent Rickets Type 2A

VDDR2A (MIM 277440), also known as hereditary vitamin D-resistant rickets, was first described by Brooks et al (34) in 1978 in a case who had skeletal findings suggesting rickets, short stature, hypocalcemia, elevated ALP, normal 25(OH)D, and very high 1,25(OH)2D. VDDR2A is an autosomal recessive disorder and is characterized by resistance to 1,25(OH)2D as a result of homozygous or compound heterozygous mutations in the vitamin D receptor gene (*VDR*, MIM 601769), which is located in 12q13.11 and consists of 11 exons. Patients with this disease usually present in infancy or early childhood, but patients with mild VDR defects may not be recognized until adolescence or adulthood (26). Clinical findings are similar to nutritional vitamin D deficiency or VDDR1A or VDDR1B except for high level of 1,25(OH)2D in VDDR2A (Table 1). Moreover, partial or total alopecia is present in many patients from birth or infancy (Figure 1) (35). The relationship between vitamin D and the hair follicle is not completely understood. However, VDR/RXRα heterodimer formation has been suggested to play an important role in the proliferation and differentiation of epidermal keratinocytes (36).

It is well known that active vitamin D mediates its biological functions by binding to its receptor VDR, which contains an N-terminal dual zinc finger DNA binding domain, a C-terminal ligand-binding domain and an extensive and

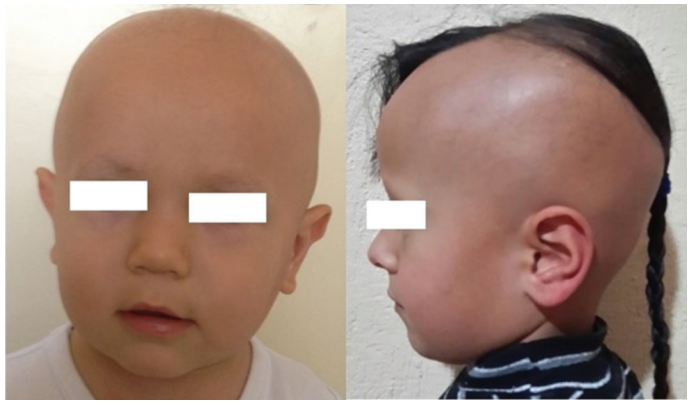

Figure 1. Near-total and partial alopecia in two children with VDDR2A (From the archives of Division of Pediatric Endocrinology, Dokuz Eylül University)

unstructured region that links the two functional domains together (15). After binding of vitamin D, VDR forms a ternary structure with RXRα, which binds to a VDRE in the promoter region of vitamin D-regulated genes to initiate transcription (37,38). Currently, there are 65 different mutations listed in HGMD (accessed Nov 13, 2017). Inactivating mutations that affect any domain of VDR would lead to disease development. Mutations in the DNA binding domain that lead to complete loss of function result in severe clinical presentations accompanied by alopecia, whereas mutations in the ligand binding domain usually cause partial loss of VDR functions and a milder phenotype without alopecia (35,38). In addition to the genotype-phenotype relationship, the clinical presentation of the disease may improve with age. Serum levels of calcium, phosphate and ALP may gradually normalize in some pubertal cases and calcitriol/calcium treatment would be unnecessary (39,40,41). Intestinal calcium absorption has been shown to become less vitamin D-dependent after the end of puberty (40).

Hypocalcemia, hypophosphatemia, increased serum levels of ALP and PTH, and normal serum levels of 25(OH)D are usually found. Hypocalcemia, hypophosphatemia and increased PTH lead to activation of 1-alpha hydroxylase and inhibition of 24-hydroxylase. Therefore, low levels of 24,25(OH)2D and high levels of 1,25(OH)2D (300-1000 pg/mL, normal range: 15-90 pg/mL) are generally present (4,26).

High doses of oral calcitriol (1-6 μg/kg/day, 2 doses) and calcium (1-3 g/day elementary calcium) are the recommended treatment (26,39). Serum calcium, phosphate, ALP and PTH levels should be intermittently monitored and regular urine calcium excretion and renal ultrasonography are suggested because of the risk of nephrocalcinosis. Clinical presentation and response to

treatment varies depending on the location of mutations in the *VDR*: patients with alopecia and nonsense mutations in the DNA-binding domain frequently exhibit a poor response to treatment (35,38). Treatment response may also be poor in patients without alopecia (42).

Long-term, high-dose intracaval/intravenous calcium (0.4-1.4 g/m^2/day) treatment is also effective (38,43,44). After successful response to the treatment regimen, it is recommended to continue with high dose oral calcium (3.5-9.0 g/m^2/day) (26,45). On the other hand, parenteral calcium therapy requires long-term hospitalization and may be associated with a number of complications such as cardiac arrhythmia, hypercalciuria, nephrocalcinosis, catheter related sepsis and extravasation of calcium (45,46). A case of VDDR2A without alopecia has been successfully treated with enteral administration of elemental calcium (calcium chloride) via gastric tube (47). Prolonged serum calcium deprivation might lead to secondary hyperparathyroidism and, if not managed properly, tertiary hyperthyroidism. Cinacalcet is reported to be effective in cases with VDDR2A and tertiary hyperparathyroidism (48,49).

1.4. Vitamin D Dependent Rickets Type 2B

VDDR2B (MIM 600785) is an unusual form of rickets due to abnormal expression of a hormone response element-binding protein that interferes with normal function of VDR. The disease was first described by Hewison et al (50) in 1993 in a patient with alopecia, skeletal abnormalities and biochemical features classically associated with VDRR2, but without *VDR* mutations (4). The similar clinical and genetic features were also found in more than 200 affected children from a rural area of southwest Colombia in 1995 (51). In contrast to VDDR2A, functions of VDR and VDR-RXR heterodimer formation are normal in VDDR2B (52). The main pathology is the overexpression of heterogeneous nuclear ribonucleoproteins (hnRNPs) C1 and C2 proteins, members of the hnRNP family, that prevent VDR-RXR heterodimer binding to VDRE (52,53). Without genetic testing, the differential diagnosis cannot be made between VDDR2A and VDDR2B (Table 1). The same treatment approaches for VDDR2A are used for patients with VDDR2B.

2. Hypophosphatemic Rickets

Hereditary HR is a group of rare, renal phosphate wasting disorders with a prevalence of 3.9 per 100,000 live births and differential diagnosis often requires genetic testing (54,55). It is characterized by renal phosphate wasting, leading to subsequent hypophosphatemia and bone mineralization defects such as rickets and osteomalacia. Hypophosphatemia and normal serum calcium are typical biochemical findings (55).

Serum levels of phosphate are maintained in the main by vitamin D and PTH. 1,25(OH)2D increases phosphate absorption from the intestine and suppresses the biosynthesis and secretion of PTH (5,56). PTH exhibits its phosphaturic effect by reducing the expression of NaPi-2a (*SLC34A1*) and NaPi-2c (*SLC34A3*) phosphate transporter in the renal tubules via PTH1R, a member of the G protein-coupled receptor family (5). In addition, several molecules [fibroblast growth factor 23 (FGF23), secreted frizzled related protein 4 (sFRP4), matrix extracellular phosphoglycoprotein, and FGF7], so-called phosphatonins, have been shown to reduce serum phosphate via direct inhibition of renal phosphate absorption in the proximal tubule (13). FGF23 and sFRP4 can also indirectly inhibit 25-OH vitamin D 1-α hydroxylase and thus intestinal phosphate absorption (57,58).

FGF23 is the most important phosphaturic agent and is produced from osteocytes and osteoblasts (57). There is a close relationship between serum phosphate and FGF23 levels. In response to elevated or decreased phosphate levels, serum FGF23 levels increase or decrease, respectively (5,58). FGF23 activates renal klotho/FGF receptor 1 (FGFR1) receptor heterodimers to inhibit renal phosphate reabsorption by down-regulation of NaPi-2a and NaPi-2c expression in the renal proximal tubules (58). FGFR3 and FGFR4 are also involved in mediating FGF23 activities (59). Klotho, a transmembrane protein, is required for FGF23 function and klotho knockout mice exhibit extremely high levels of serum FGF23, most likely due to end-organ resistance to FGF23 (60,61). In addition, FGF23 inhibits 25-OH vitamin D 1-α hydroxylase and activates 25-OH vitamin D 24-hydroxylase, resulting in decreased 1,25(OH)2D and increased 24,25(OH)2D levels (62).

Another molecule that plays a role in phosphate regulation is sodium-hydrogen exchanger regulatory factor 1 (NHERF1) (58). NHERF1 has been shown to have two different effects on phosphate reabsorption in the proximal tubules. The first is to bind to PTH1R to reduce the effect of PTH-induced cAMP synthesis and the second is to increase the activation of NaPi-2a by interacting with C-terminal region of the protein (58,62).

Serum phosphate levels normally vary according to age, which needs to be carefully considered when assessing whether hypophosphatemia is present or not. Normal ranges of serum phosphate are 4.8-8.2 mg/dL for 0-5 days of age, 3.8-6.5 mg/dL for 1-3 years of age, 3.7-5.6 mg/dL for 4-11 years of age, 2.9-5.4 mg/dL for 12-15 years of age and 2.7-4.7 mg/dL for 16-19 years of age (27). In addition to hypophosphatemia, decreased TRP, normal or mildly elevated serum levels of PTH and markedly elevated serum levels of ALP are typically detected. In a study comparing serum levels of ALP and PTH in HR, VDDR and nutritional

rickets, the highest serum levels of PTH and ALP have been found in patients with VDDR and the lowest levels in patients with HR (63).

Renal phosphate excretion can be evaluated using various parameters. The most widely used is the TRP defined by the formula: 1-(urine phosphate x serum creatinine) / (serum phosphate x urine creatinine). Various lower limits for TRP are generally used in daily practice ranging from 75-85%. However, in the presence of hypophosphatemia, fractional excretion of filtered phosphate should be less than 5% (TRP >95%) (64). The ratio of tubular maximum reabsorption rate of phosphate per glomerular filtration rate (TmP/GFR) is a superior method for assessing phosphaturia, which can be assessed via the nomogram of Walton and Bijvoet or can be calculated as shown below:

For TRP ≤86%: TmP/GFR = TRP x serum phosphate

For TRP >86%: TmP/GFR = (0.3 x TRP) / [1-(0.8 x TRP)] x serum phosphate

Low TmP/GFR values in the setting of hypophosphatemia points to renal phosphate wasting (65). The normal ranges of TmP/GFR (mg/dL) vary with age: Birth, 3.6-8.6; 3 months of age, 3.7-8.25; 6 months of age, 2.9-6.5; 2-15 years of age, 2.9-6.1, and the normal adult range for TmP/GFR is 2.2 to 3.6 mg/dL (66).

Laboratory findings such as normal serum calcium, low serum phosphate and elevated serum ALP and PTH may not always be diagnostic of HR. These can also be seen in rickets (especially in stage 2) associated with vitamin D deficiency or disorders of vitamin D biosynthesis (20). The distinctive finding is that PTH is significantly higher in vitamin D-related rickets, whereas normal/mildly elevated PTH is expected in HR (26). To date, a variety of genetic causes leading to HR have been identified (Table 2) (5,58,62). Some of these genetic defects lead to an increase in serum FGF23 levels (FGF23-related or -dependent HR), while others affect phosphate transporters which does not affect serum FGF23 levels (FGF23-independant HR). Laboratory characteristics of several types of HR are summarized in Table 3.

2.1. FGF23-Related Hypophosphatemic Rickets

2.1.1. X-linked Dominant Hypophosphatemic Rickets

X-linked dominant HR (XLDHR, MIM 307800) is the most common type of HR with an incidence of approximately 1 in 20000 live births and is caused by inactivating mutations of *PHEX* (phosphate regulating gene with homologies to endopeptidases on the X chromosome, MIM 307800) (55,67). XLDHR affects both genders equally in terms of disease severity as a result of random X-inactivation in girls (62). Skeletal findings of the disease frequently appear in the late infantile period and are especially evident by the effect on body weight in the period after starting to walk (5). *PHEX* encodes a membrane endopeptidase, which is expressed in mature osteoblasts and odontoblasts, and plays a role in down-regulation of FGF23 expression (68). Therefore *PHEX* mutations would lead to increased serum levels of FGF23 (69). Currently, there are 423 *PHEX* mutations listed in HGMD (accessed Nov 13, 2017).

In the Turkish population, *PHEX* mutation is also the most common cause of HR, accounting for 87% cases (55,70,71). *De novo* mutations are frequent and more often occur in female patients, likely resulting from mutagenesis of the X chromosome in paternal germ cells (70).

Typical clinical findings include short stature, wrist enlargement, rachitic rosary, bowed legs, frontal bossing, dental abscess and bone pain in children. Osteomalacia, bone pain, dental abscess and spinal canal stenosis are typical presentation in adult patients. Laboratory findings include low serum levels of phosphate, decreased TRP, normal/mildly elevated PTH and high levels of ALP with normal calcium and 25(OH)D, and inappropriately normal or low serum 1,25(OH)2D levels (Table 3). These clinical and laboratory findings suggest HR but confirmation of diagnosis requires genetic confirmation of *PHEX* mutations.

2.1.2. Autosomal Dominant Hypophosphatemic Rickets

Autosomal dominant HR (ADHR, MIM 193100) is caused by gain-of-function mutations in the proteolytic cleavage domain of FGF23 (R176XXR179, MIM 605380). Mutations that alter the arginine (R) residue at the position 176 or 179 would render the protein resistant to proteolytic cleavage and lead to increased serum levels of FGF23 and its activity, resulting in hypophosphatemia (61,71,72). It is less common than XLHR and 16 different mutations are reported in HGMD (accessed Nov 13, 2017).

ADHR exhibits similar clinical and laboratory findings as XLHR and also needs genetic testing for diagnosis. Differences in the age of onset, severity and a waxing and waning course of phosphate wasting (renal phosphate wasting can be spontaneously normalized) is related to serum FGF23 levels (73,74). This led to the discovery that iron deficiency is an environmental trigger, which stimulates FGF23 expression and thus hypophosphatemia in ADHR (75,76,77).

2.1.3. Autosomal Recessive Hypophosphatemic Rickets

2.1.3.1. Autosomal Recessive Hypophosphatemic Rickets Type 1

ARHR type 1 (ARHR1, MIM 241520) is due to inactivating homozygous mutations in the *DMP1* gene (dentin matrix acidic phosphoprotein 1, MIM 600980) (78). DMP1 is an

Table 2. Genetic causes of hypophosphatemic rickets

Disease	Abbreviation	Gene	Protein	İnheritance	Clinical characteristics
			FGF23-dependent HR		
X-linked dominant hypophosphatemic rickets	XLDHR	*PHEX*	Phosphate regulating endopeptidase	X-linked dominant	Increased FGF23, decreased renal phosphorous reabsorption
Autosomal dominant hypophosphatemic rickets	ADHR	*FGF23*	Fibroblast growth factor 23	AD	
Autosomal recessive hypophosphatemic rickets Type 1	ARHR1	*DMP1*	dentin matrix acidic phosphoprotein 1	AR	
Autosomal recessive hypophosphatemic rickets Type 2	ARHR2	*ENPP1*	Ectonucleotide pyrophosphatase / phosphodiesterase 1	AR	
Hypophosphatemic rickets with hyperparathyroidism	HRHPT	9:13 balanced translocation affecting *KL* gene	α-klotho	unknown	Increased alpha-klotho and FGF23 levels and beta-glucuronidase activity. Hypercalciuria, nephrocalcinosis, parathyroid hyperplasia
Osteoglophonic dysplasia		*FGFR1*	Fibroblast growth factor receptor 1	AD	Craniofacial abnormalities, increased FGF23
McCune-Albright Syndrome		*GNAS*	Guanine nucleotide binding protein, alpha	Postzygotic somatic mutation	Fibrous dysplasia, increased FGF23
Raine syndrome		*FAM20C*	Famıly wıth sequence similarity 20, member c (FAM20C)	AR	Generalized osteosclerosis, increased FGF23
Opsismodysplasia		*INPPL1*	Inositol polyphosphate phosphatase-like 1	AR	Craniofacial abnormalities, increased FGF23
			FGF23-independent HR		
Hereditary HR with Hypercalciuria	HHRH	*SLC34A3*	Sodium-dependent phosphate transport protein 2C	AR	Hypercalciuria, hypophosphatemia, nephrocalcinosis
Hypophosphatemic rickets with nephrolithiasis and osteoporosis type 1 Infantile hypercalcemia Type 2; Fanconi renotubular syndrome Type 2	NPHLOP1 HCINF2 FRTS2	*SLC34A1*	Sodium-dependent phosphate transport protein 2A	AD, AR	Hypercalciuria, hypophosphatemia, nephrocalcinosis, proximal tubulopathy
Hypophosphatemic rickets with nephrolithiasis and osteoporosis type 2	NPHLOP2	*SLC9A3R1*	Sodium-hydrogen exchanger regulatory factor 1 (NHERF1)	AD	Hypercalciuria, nephrocalcinosis and decreased bone mineral density
Dent disease 1		*CLCN5*	Chloride Voltage-Gated Channel 5	X-linked, recessive	Hypercalciuria, hypophosphatemia, nephrocalcinosis, renal failure, proteinuria, and glucosuria
Dent disease 2 or Lowe syndrome		*OCRL1*	Inositol Polyphosphate-5-Phosphatase	X-linked recessive	Mild mental retardation, developmental delay, hypophosphatemia, hypercalciuria, nephrocalcinosis, amino aciduria, and proteinuria

AD: autosomal dominant, AR: autosomal recessive, *FGF23*: Fibroblast growth factor 23, *PHEX*: Phosphate regulating endopeptidase homolog x-linked, XLDHR: X-linked dominant hypophosphatemic rickets, ADHR: Autosomal dominant hypophosphatemic rickets, ARHR1: Autosomal recessive hypophosphatemic rickets Type 1, *DMP1*: Dentin matrix acidic phosphoprotein, *ENPP1*: Ectonucleotide pyrophosphatase/phosphodiesterase 1, *FGFR1*: Fibroblast growth factor receptor 1, *INPPL1*: Inositol polyphosphate phosphatase-like 1, *CLCN5*: Chloride voltage-gated channel 5

Tale 3. Laboratory characteristics of genetic causes of hypophosphatemic rickets

Disease	Gene	FGF23	TmP/GFR	Serum calcium	Serum phosphate	ALP	PTH	1,25 (OH)2D	Urinary calcium/creatinine
FGF23-dependent HR									
X-linked dominant HR	*PHEX*	↑ or N	↓	N	↓	↑	N or ↑	N or ↓	N
Autosomal dominant HR	*FGF23*	↑ or N	↓	N	↓	↑	N or ↑	N or ↓	N
Autosomal recessive HR Type 1	*DMP1*	↑ or N	↓	N	↓	↑	N or ↑	N or ↓	N
Autosomal recessive HR Type 2	*ENPP1*	↑ or N	↓	N	↓	↑	N or ↑	N or ↓	N
Osteoglophonic dysplasia	*FGFR1*	↑ or N	↓ or N	N	↓ or N	↑ or N	N or ↑	N or ↓	N
McCune-Albright Syndrome	*GNAS*	↑ or N	↓ or N	N	↓ or N	↑ or N	N or ↑	N or ↓	N
Raine syndrome	*FAM20C*	↑ or N	↓ or N	N	↓ or N	↑ or N	N or ↑	N or ↓	N
Opsismodysplasia	*INPPL1*	↑ or N	↓ or N	N	↓ or N	↑ or N	N or ↑	N or ↓	N
Hypophosphatemic rickets with hyperparathyroidism	9:13 balanced translocation affecting *KL* gene	↑	↓	N or ↑	↓	↑	↑	N	N
FGF23-independent HR									
Hereditary HR with Hypercalciuria	*SLC34A3*	↓ or N	↓	N	↓	N or ↑	N	↑	↑
Hypophosphatemic rickets with nephrolithiasis or osteoporosis Type 1 Infantile hypercalcemia Type 2 Fanconi renotubular syndrome Type 2	*SLC34A1*	↓ or N	↓	N or ↑	↓	N or ↑	N or ↓	↑	↑
Hypophosphatemic rickets with nephrolithiasis and osteoporosis Type 2	*SLC9A3R1*	↓ or N	↓	N	↓	↑	N or ↓	↑	↑
Dent Disease 1	*CLCN5*	↓ or N	↓	N	↓	↑	N or ↓	↑	↑
Dent Disease 2 or Lowe syndrome	*OCRL1*	↓ or N	↓	N	↓	↑	N or ↓	↑	↑

ALP: alkaline phosphatase, PTH: Parathyroid hormone, N: normal, *FGF23*: Fibroblast growth factor 23, *PHEX*: Phosphate regulating endopeptidase homolog x-linked, *DMP1*: Dentin matrix acidic phosphoprotein, *ENPP1*: Ectonucleotide pyrophosphatase/phosphodiesterase 1, *INPPL1*: Inositol polyphosphate phosphatase-like 1, *FGFR1*: Fibroblast growth factor receptor 1, *FAM20C*: Family with sequence similarity 20, member c, *CLCN5*: Chloride voltage-gated channel 5, 1,25(OH)2D: 1,25-dihydroxyvitamin D, *GFR*: Growth factor receptor

extracellular matrix protein expressed in osteoblasts and osteocytes and acts in the inhibition of FGF23 expression (62,68). Inactivating mutations of *DMP1* result in an increase in serum FGF23 levels and thus leads to HR. Clinical, laboratory and radiological findings are similar to those of XLHR and ADHR. There are 9 different mutations listed in the HGMD (accessed Nov 13, 2017). *DMP1* knockout mice have displayed increased serum levels of FGF23, hypophosphatemia, skeletal and dental anomalies and osteomalacia (79). Unlike other HR types, osteosclerosis in the base of skull and calvarial bones may occur (62). Haploinsufficiency has been reported in heterozygous carriers: mild hypophosphatemia, low TRP and focal osteomalacia, without typical skeletal deformities of rickets (80).

2.1.3.2. Autosomal Recessive Hypophosphatemic Rickets Type 2

ARHR type 2 (ARHR2, MIM 613312) is caused by inactivating homozygous mutations in ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1, MIM 173335) (81).

Interestingly, the majority of *ENPP1* mutations (49 mutations) have been reported in patients with idiopathic infantile arterial calcification or generalized arterial calcification of infancy, which is an autosomal recessive disorder and characterized by calcification of the internal elastic lamina of muscular arteries and stenosis due to myointimal proliferation (82). There are only eight mutations reported in patients with HR (HGMD, accessed Nov 13, 2017), suggesting a different pathway is involved in the generation of ARHR2 (83).

By generating inorganic pyrophosphate (PPi), ENPP1 plays an important role in the regulation of pyrophosphate levels, bone mineralization and soft tissue calcification. The mineral accumulation in the bones is determined by the ratio of phosphate and PPi that is balanced by ENPP1 (84). Enpp1 knockout mice show altered bone development and an increase in FGF23 expression (84). *ENPP1* mutations increase serum levels of FGF23. However, the mechanism of FGF23 elevation caused by ENPP1 mutation is not completely understood (82,83,84).

2.1.4. Hypophosphatemic Rickets with Hyperparathyroidism

HR with hyperparathyroidism (MIM 612089) is a very rare disease caused by a balanced translocation with breakpoints at 9q21.13 and 13q13.1, which is adjacent to the *KL* gene (85). Its product, alpha-Klotho, is implicated in aging and regulation of FGF signaling and calcium homeostasis (86). The translocation result in increased serum α-klotho, FGF23 levels and β-glucuronidase activity (85). The disease is characterized by hypophosphatemia and elevated serum PTH levels, with inappropriate renal phosphate wasting (85). Increased levels of FGF23 lead to decreased TRP, hypophosphatemia and rickets. Hyperparathyroidism due to diffuse parathyroid hyperplasia results in increased levels of PTH. It is not clear whether increased levels of α-klotho cause parathyroid hyperplasia. PTH levels in this disease are much higher compared to other causes of HR and are comparable with those in VDDR. Klotho knockout mice, deficient for α-klotho, display a phenotype comparable with human ageing and are characterized by a mild hypercalcemia, hyperphosphatemia, increased levels of serum 1,25(OH)2D, decreased PTH and bone abnormalities such as increased metaphyseal trabecular bone mass and soft tissue calcifications, which are different from the phenotype caused by the translocation [hypophosphatemia, high PTH, and normal 1,25(OH)2D7] (87,88). Treatment includes calcitriol with oral phosphate supplementation.

2.1.5. Other Genetic Causes

2.1.5.1. Osteoglophonic Dysplasia

Osteoglophonic dysplasia (MIM 166250) is caused by heterozygous gain-of-function mutations in *FGFR1* (MIM 136350), a rare autosomal dominant disorder characterized by craniosynostosis, rhizomelic short stature, maxillary hypoplasia, depressed nasal bridge, mandibular pragmatism, dental anomalies, tower-shaped skull, vertebral anomalies and bone mineralization defects (metaphyseal radiolucent changes) (89). High levels of serum FGF23, low levels of serum phosphate and 1, 25(OH)2D, and low TRP are present in some patients (89). Increased FGF23 leads to renal phosphate wasting, hypophosphatemia and deterioration of bone mineralization. It has been suggested that FGF23 production is stimulated from bone tissue due to the effect of activating mutations in *FGFR1* (5). Among 197 mutations in *FGFR1*, only three are reported in patients with osteoglophonic dysplasia (HGMD, accessed Nov 13, 2017).

2.1.5.2. McCune-Albright Syndrome

McCune-Albright Syndrome (MAS, MIM 174800) is caused by post-zygotic activating mutations in the Gsα subunit of G proteins (encoded by *GNAS*, MIM 139320), leading to a mosaic distribution of cells bearing constitutively active adenyl cyclase activity. The disease is characterized by the classic triad of polyostotic fibrous dysplasia, cafe-au-lait skin pigmentation and peripheral precocious puberty, but is clinically heterogeneous and usually include hyperfunctional endocrinopathies such as thyrotoxicosis, pituitary gigantism and Cushing syndrome due to autonomous hormonal hyper-production (90). There is an association between fibrous dysplasia of bone tissue and increase in serum FGF23 level. TRP is decreased in 50% of cases (91). Therefore, hypophosphatemic rickets/osteomalacia can be seen in these patients. More than 250 mutations are listed in the HGMD (accessed Nov 13, 2017) and most of them (221 inactivating mutations) are found in patients with resistance to PTH (pseudohypoparathyroidism or Albright hereditary osteodystrophy, which is different from the disease). In all patients reported to date, there are only two activating mutations (p.R201H or p.R201C and p.T55A) listed in the HGMD (accessed Nov 13, 2017) that is associated with McCune-Albright Syndrome.

2.1.5.3. Raine Syndrome

Raine syndrome (MIM 259775) is an autosomal recessive disorder first described in 1989 by Raine et al (92) in a case with generalized osteosclerosis of the periosteal bone formation and severe craniofacial dysmorphology. The disease is caused by mutations in the *FAM20C* (family with sequence similarity 20, member c, also called dentin matrix protein 4 DMP4; MIM 611061) and was initially reported to be lethal (93). Non-lethal cases have since been found (94). *FAM20C* is mainly expressed in osteoblasts, odontoblasts and ameloblasts in skeletal and dental tissues and is a

novel FGF23 regulator (95,96). Increased renal phosphate loss and hypophosphatemia due to increased serum FGF23 levels have been reported in Raine's syndrome (97,98,99). HR has been observed in *FAM20C* knockout mice (96). FAM20C can suppress FGF23 production by enhancing DMP1 expression and its inactivation causes FGF23-related hypophosphatemia by decreasing transcription of DMP1, resulting in increased FGF23 levels in patients with Raine's syndrome (98). There are 22 mutations listed in the HGMD (accessed Nov 13, 2017).

2.1.5.4. Opsismodysplasia

Opsismodysplasia (OPSMD, MIM 258480) is a rare skeletal dysplasia involving delayed bone maturation first described by Zonana et al (100) in 1977 and later defined by Maroteaux et al (101) in 1982. It is an autosomal recessive disease and caused by mutations in the *INPPL1* gene (inositol polyphosphate phosphatase-like 1, MIM 600829) (102). Clinical signs observed at birth include short limbs, small hands and feet, relative macrocephaly with a large anterior fontanelle and characteristic craniofacial abnormalities such as a prominent brow, depressed nasal bridge, a small anteverted nose and relatively long philtrum. Abdominal protrusion, abnormalities of the extremities, progressive bone demineralization, delayed bone maturation and hypotonia are commonly reported (103). The main radiological features are severe platyspondyly, short long bones including squared metacarpals, delayed epiphyseal ossification, and metaphyseal flaring and cupping (103). In addition to these clinical and radiological findings, increased renal phosphate excretion and HR have been reported by Zeger et al (104). The serum level of FGF23 was high in one of the two patients at three years of age. Currently, there are 26 mutations listed in the HGMD (accessed Nov 13, 2017).

2.1.6. Treatment of FGF23-related Hypophosphatemic Rickets

There is no difference in the management of XLHR, ADHR, ARHR and other rare genetic causes of HR. It is a lifelong treatment of phosphate and calcitriol replacement to restore bone mineralization and improve skeletal deformities. Calcitriol is recommended at doses ranging from 25 to 70 ng/kg/day (2 doses) and elemental phosphate at 30 to 70 mg/kg/day (4-6 doses) (26). The main goal of treatment is to achieve low-normal serum phosphate and high-normal serum ALP levels (105). Treatment should not attempt to normalize serum phosphate levels by giving aggressive phosphate therapy as this might lead to side effects such as diarrhea, secondary hyperparathyroidism, increased FGF23 synthesis, nephrocalcinosis and renal insufficiency (105). In addition, serum phosphate levels should not be used alone in evaluating response to treatment, due to rapid fluctuations in serum levels. Therefore, reduction in ALP levels, improvement in clinical findings and growth velocity after treatment are more useful indicators in assessing treatment response. Traditional calcitriol and phosphate therapy improves bone mineralization, skeletal findings of rickets and growth rate. However, despite these treatments, skeletal deformities may persist to varying degrees in some patients (105).

Phosphate salts (sodium phosphate, potassium phosphate) are generally used for phosphate replacement. It can be given in tablet or solution form both of which are equally effective. Tablet form (Phosphate-Sandoz®) contains a high dose of phosphate supplement, consisting of sodium phosphate monobasic. Each tablet provides elemental phosphate 500 mg (16.1 mmol phosphate), sodium 469 mg (20.4 mmol Na^+), potassium 123 mg (3.1 mmol K^+) and citric acid-anhydrous 800 mg. "Joulie's solution" can be used for children if the tablet form is not available. Prepared with 136 g of dibasic sodium phosphate, 58.8 g phosphoric acid and 1000 mL of distilled water, 1 mL of this solution contains 30.4 mg of elemental phosphate (106). More frequent dividing of phosphate dose avoids a profound drop in post-dose serum phosphate levels and reduces the frequency of diarrhea, the most common side effect of this treatment.

Patients should be monitored for clinical, anthropometric and laboratory characteristics at three month intervals. Laboratory assessments include serum calcium, phosphate, ALP and PTH levels, as well as urinary calcium and creatinine for hypercalciuria. In addition, renal ultrasonography should be performed annually, before and after treatment, to monitor the development of nephrocalcinosis (105). Skeletal X-ray is recommended to be performed annually before treatment and during treatment for monitoring of skeletal findings (5).

The dosage of calcitriol should be adjusted according to serum levels of PTH and the urine calcium/creatinine ratio. The main goal is to suppress PTH, maintain serum calcium in the normal range and prevent hypercalciuria. Twenty-four hours of urinary calcium excretion above 4 mg/kg/day indicates increased calcium excretion (hypercalciuria) (26). In addition, the ratio of calcium to creatinine in the spot urine can be used. The normal range varies with age: ≤6 months of age, <0.8; 7-12 months of age, <0.6; 1-3 years of age, <0.53; 3-5 years of age, <0.39; 5-7 years of age, <0.28; >7 years of age, <0.21 (28). In the presence of hypercalciuria, it is necessary to reduce calcitriol dosage. The evening dosage of calcitriol should be higher in order to suppress increased secretion of PTH at night (26).

There is a close relationship between high dose phosphate therapy and the development of nephrocalcinosis (107,108). The frequency of nephrocalcinosis in HR patients after calcitriol and phosphate combined therapy is between 33% and 80%, and usually occurs within the first 3-4 years of treatment (105,107,108,109). However, long-term follow-up of cases with nephrocalcinosis has been reported to have no significant impairment on renal function (110). On the other hand, long-term, high-dose phosphate therapy may result in secondary and tertiary hyperparathyroidism (105,111,112,113). Cinacalcet can be used in the treatment of tertiary hyperparathyroidism in children with HR (111). In brief, oral phosphate should be given at the lowest dose that is sufficient to improve rickets and patients should be monitored for the development of hyperparathyroidism and nephrocalcinosis.

Conventional treatment should gradually improve biochemical and skeletal abnormalities, however mild or moderate skeletal deformities may persist in some patients. For these patients, some devices, such as braces, are suggested to correct leg bowing. If such devices are not tolerated, surgical correction can be considered. In children younger than 10 years with XLHR, femoral and tibial hemiepiphysiodesis are recommended to correct lower extremity deformities, which is a relatively minor surgical procedure to allow appropriate growth (114). For children older than 10 years of age, osteotomy is suggested, a surgical procedure in which a surgeon removes a wedge of bone near a damaged joint (26).

Short stature is one of the major findings in the diagnosis of HR patients. With appropriate calcitriol and phosphate treatment, the skeletal and biochemical findings should improve and an increase in height velocity should be achieved. However, some patients with XLHR do not achieve the desired height velocity despite appropriate treatment (115,108). It is suggested that this may be related to delayed treatment or deficit in GH secretion (115,116). Recombinant human growth hormone (rhGH) treatment, especially in the pre-pubertal period, has been demonstrated to significantly increase height velocity and positively contributes to final height in these patients (117,118,119).

Recent progress in treatment has focused on the pathogenesis of HR. It has been shown that pharmacological inhibition of FGF receptor signaling ameliorates FGF23-mediated HR using NVP-BGJ398, a novel, selective, FGFR inhibitor that inhibits FGFR1, FGFR2, and FGFR3 with IC50 of 0.9 nM, 1.4 nM, and 1 nM, respectively (120). Similar results have been achieved using anti-FGF23 antibody (KRN23), a human monoclonal KRN23 (121). In a study of 28 adults with XLHR who received monthly KRN23, a significant increase in serum phosphate, 1,25(OH)2D and maximum renal tubular threshold for phosphate reabsorption (TmP/GFR) has been observed after four or twelve months of treatment (121). The half-life is 8-12 days after intravenous administration and longer (13-19 days) after subcutaneous administration. The serum levels of phosphate remained higher than baseline level for four weeks (122,123). Therefore, it is recommended that KRN23 should be given at four weekly intervals. Finally, phase III studies of KRN23 in adults and children are still ongoing.

2.2. Hypophosphatemic Rickets Accompanied by Hypercalciuria (FGF23-independent Rickets)

2.2.1. Hereditary Hypophosphatemic Rickets with Hypercalciuria

Hereditary HR with hypercalciuria (HHRH, MIM 241530) is an autosomal recessive disease caused by inactivating mutations in the *SLC34A3* (solute carrier family 34, member 3, also known as NaPi-2c, MIM 609826) (124). *SLC34A3* plays a role in phosphate reabsorption in the kidney and its mutation results in increased renal phosphate loss and subsequent hypophosphatemia (5). FGF23 is not involved in the disease. The decrease in serum phosphate promotes biosynthesis of 1,25(OH)2D, which leads to increase in the absorption of intestinal calcium, suppressed PTH and development of hypercalciuria and nephrocalcinosis. Diagnosis can be made based on skeletal findings of rickets, hypophosphatemia, hypercalciuria and nephrolithiasis (124,125). There are 33 mutations listed in HGMD (accessed Nov 13, 2017) and genotype-phenotype correlation has not yet been established (125,126,127). Increased renal phosphate wasting, mild hypophosphatemia, increased 1,25(OH)2D and hypercalciuria without metabolic bone disease, can be present in patients with heterozygous *SLC34A3* mutations, indicating haploinsufficiency (124).

Oral phosphate alone is sufficient for patients with HHRH in contrast to patients with XLHR, ADHR or ARHP, who are usually treated with high doses of alphacalcidol or calcitriol and multiple daily doses of oral phosphate, low-sodium diet and hydration are recommended for the disease (5,26). The response to treatment is excellent. Phosphate treatment results in a decrease in serum levels of calcitriol and, consequently, urinary calcium excretion gradually returns to normal. The use of calcitriol is contradictory and harmful because it can increase hypercalciuria.

2.2.2. Hypophosphatemic Rickets with Nephrolithiasis and Osteoporosis Type 1

SLC34A1 (solute carrier family 34, member 1, MIM 182309) encodes NaPi-2a, which plays an important role in phosphate

reabsorption from proximal tubules and is down-regulated by PTH and FGF23 (128). Inactivating mutations in *SLC34A1* can cause three different diseases: HRs with Nephrolithiasis and Osteoporosis type 1 (NPHLOP1, MIM 612286) (129,130), Fanconi Renotubular Syndrome type 2 (FRTS2, MIM 613388) (131) and Infantile Hypercalcemia type 2 (HCINF2; MIM 616963) (132). NPHLOP1 was originally reported as an autosomal-dominant disease. However, multiple groups later questioned a single heterozygous mutation in the pathogenesis of the disease (131,133,134). The initial cases caused by heterozygous *SLC34A1* mutations are probably represent a milder phenotype characterized by increased renal phosphate wasting, hypercalciuria, osteoporosis and nephrolithiasis in adults. Currently, there are 25 different mutations listed in the HGMD (accessed Nov 13, 2017).

Similar to HHRH, NPHLOP1 is characterized by hypophosphatemia and decreased renal phosphate absorption with an appropriate elevation in serum 1,25(OH)2D. Laboratory findings include decreased TRP, hypophosphatemia, hypercalcemia, elevated serum 1,25(OH)2D, decreased serum PTH, hypercalciuria and nephrocalcinosis.

The original patients with FRTS2 were adults with clinical features of increased renal phosphate and other substance wasting (without loss of bicarbonate) and significantly increased 1,25(OH)2D leading to severe skeletal deformities (HR in children and osteomalacia in adults), bone pain, marked hypercalciuria, glycosuria, generalized aminoaciduria and tubular proteinuria without renal tubular acidosis (135).

HCINF2 is characterized by severe hypercalcemia with failure to thrive, vomiting, dehydration and medullary nephrocalcinosis. Laboratory findings include decreased TRP, hypophosphatemia, hypercalcemia, elevated 1,25(OH)2D, suppressed PTH, hypercalciuria, nephrocalcinosis, hyperuricosuria and low-molecular-weight proteinuria (136).

The main pathogenesis of all three diseases is increased phosphate wasting due to inactivated phosphate cotransporter NaPi-2a in the proximal tubules. They should be considered as one disease with different clinical presentations, probably caused by differences in severity of mutations. The mechanism for renal tubulopathy is unclear at present.

Treatment is the same as in HHRH. Oral phosphate replacement will result in improvement in bone pain, muscle strength and radiologic signs of rickets, with normalization of urinary calcium excretion and significant decrease in 1,25(OH)2D. However, the glomerular filtration rate, serum uric acid levels and rate of urinary excretion of glucose, protein and amino acids will remain unchanged.

2.2.3. Hypophosphatemic Rickets with Nephrolithiasis and Osteoporosis Type 2

HRs with Nephrolithiasis and Osteoporosis type 2 (Nephrolithiasis/osteoporosis, hypophosphatemic, 2, NPHLOP2, MIM 612287) is an autosomal dominant disease caused by mutations in the *SLC9A3R1* (MIM 604990). It encodes NHERF1, an adaptor protein that regulates several G protein-coupled receptors, including the PTH1R (58,137). It regulates phosphate reabsorption in the renal proximal tubules by binding to renal phosphate transporter NaPi-2a to maintain correct expression at the apical domain of proximal tubular cells and PTH1R leading to a decrease in PTH-induced cAMP synthesis and phosphate transport (128,138). Mutations in the *NHERF1* result in reduced NaPi-2a expression and hypophosphatemia due to increased renal phosphate loss. Characteristic clinical features include hypophosphatemia, hypercalcemia, elevated serum levels of 1,25(OH)2D, hypercalciuria, decreased TRP or low TmP/GFR value and nephrolithiasis, which cannot be distinguished from HHRH or NPHLOP1 without molecular testing. Serum levels of PTH and FGF23 are normal. Osteopenia has been demonstrated in patients with *NHERF1* mutations, although rickets has not yet been reported, probably reflecting late-onset and milder phenotype caused by the gene mutation. There are only four different mutations listed in the HGMD (accessed Nov 13, 2017).

2.2.4. Dent Disease

Dent disease can be divided into type 1 and type 2. Dent disease 1 (MIM 300009, also known as X-linked nephrolithiasis, X-linked nephrolithiasis type 2 (NPHL2), X-linked recessive nephrolithiasis with renal failure, or X-linked recessive nephrolithiasis type 1 (NPHL1), MIM 310468) is an X-linked recessive disease caused by mutations in the *CLCN5* gene which encodes chloride voltage-gated channel 5 (MIM300008) (139). It is characterized by proximal tubular dysfunction and 30-80% of patients can progress to chronic kidney disease or renal failure: low molecular weight proteinuria, hypercalciuria, glycosuria, phosphaturia, aminoaciduria, uricosuria, hematuria and nephrocalcinosis (140,141,142). More than 259 different *CLCN5* mutations are listed in the HGMD (accessed Nov 13, 2017). The presence of hypophosphataemic rickets in Dent disease is variable from 30-50% in patients from US and UK, to rare in Japanese patients (142,143,144). Clinical presentations and *CLCN5* mutations are heterogeneous and there is no genotype-phenotype correlation.

Dent disease 2 (MIM 300555, or Lowe syndrome or oculocerebrorenal syndrome, MIM 309000) is also an X-linked recessive disease caused by mutations in the *OCRL*

gene (MIM 300535) which encodes inositol polyphosphate-5-phosphatase (145). Clinical features are similar to Dent disease 1 and genetic testing is required to distinguish between them. There is a broad phenotypic spectrum of OCRL mutations and Dent disease 2 may be a mild variant of Lowe syndrome characterized by hydrophthalmia, cataract, mental retardation, HR, amino aciduria, proteinuria and phosphaturia (146).

There are 245 different *OCRL* mutations listed in the HGMD (accessed Nov 13, 2017). Approximately 50-60% of cases with Dent disease have *CLCN5* mutations, 15-20% have *OCRL* mutations and the remaining cases have no detectable mutation (140,146). Patients usually respond well to oral phosphate for the treatment of hypophosphatemia. In addition, some patients may need calcitriol, but it should be carefully used as it may increase urinary calcium excretion. A sodium-restricted diet to reduce urinary calcium excretion may be useful.

Conclusion

Calcium and phosphate, which play important roles in bone mineralization, are regulated by various molecules such as PTH, 1,25(OH)2D and FGF23. Nutritional vitamin D deficiency is the most common cause of rickets due to low vitamin D in breast milk, social and economic conditions that prevent access to vitamin D from other sources, or climatic conditions preventing adequate ultraviolet light exposure. Various genetic causes of rickets should be considered to avoid delay in diagnosis and treatment. Rickets caused by calcium deficiency should also be considered, which usually occurs among older toddlers and children due to low dietary calcium intake. Although clinical presentations are usually similar, differential diagnosis of different types of rickets such as nutritional and VDDR (VDDR1A, VDDR1B, VDDR2A and VDDR2B) can be made by examining serum levels of 25(OH)2D and 1,25(OH)2D, and their responses to treatment (calcium, vitamin D or calcitriol) (Table 1).

The genetic causes of HR can be divided into two groups: FGF23-dependent and FGF23-independent groups (Table 2). The most common genetic cause of HR is XLDHR resulting from *PHEX* mutations. Although clinical presentations are similar, differential diagnosis between these two groups can be made by serum FGF23 levels. However, diagnosis of individual diseases within each group often require molecular testing to confirm diagnosis. The current treatment for FGF23-dependant HR is oral phosphate replacement and calcitriol which have potential treatment complications such as calciuria and nephrocalcinosis. Recent progress of targeted therapy against FGF23-mediated HR (NVP-BGJ398 and KRN23) has produced promising results and may offer better therapeutic outcome in the future. In the FGF23-independent HR group, hypercalciuria and nephrolithiasis are major clinical findings and oral phosphate replacement alone is sufficient in the treatment. Furthermore, there are some HR patients whose genetic defects remain to be identified.

Ethics

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Authorship Contributions

Concept: Sezer Acar, Korcan Demir, Yufei Shi, Design: Sezer Acar, Korcan Demir, Yufei Shi, Data Collection or Processing: Sezer Acar, Korcan Demir, Yufei Shi, Analysis or Interpretation: Sezer Acar, Korcan Demir, Yufei Shi, Literature Search: Sezer Acar, Korcan Demir, Yufei Shi, Writing: Sezer Acar, Korcan Demir, Yufei Shi.

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REVIEW

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Sex Assignment in Conditions Affecting Sex Development

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Abstract

The newborn infant with atypical genitalia presents a challenging clinical scenario and requires expert input. There have been appreciable advances in our knowledge of the underlying causes that may lead to a mere difference or a more serious disorder of sex development (DSD), the natural history of conditions, as well as the short and long-term complications of these conditions themselves, together with the clinical interventions that are associated with these conditions. With this information, the DSD expert can be more confident when discussing options with the parents of the newborn infant. By working within a multidisciplinary team, the expert should be able to support the family whilst individualising the management plan so that it is also cognizant of the shifts in societal attitudes and expectations around concepts of diversity and openness. It is, therefore, likely that the practice of assigning sex, especially in those cases where sex assignment is unclear on expert assessment, will continue to show temporal, social and geographical variations. It is imperative that clinical data for rare conditions such as these are collected in a standardized format and shared through a common registry so that any evidence that is used for future shifts in practice has a stronger foundation than that which is currently available.
Keywords: Atypical, ambiguous, disorder of sex development, genitalia

Introduction

When sex development is affected in early life, the involved infant often presents with atypical genitalia in the neonatal period. This presentation raises the possibility of a disorder of sex development (DSD) (1). The underlying biological condition in a number of cases of atypical genitalia, especially those with a 46 XY karyotype who are raised as a boy, remains unclear. The newborn infant that has genitalia that are so atypical that a diagnosis cannot be reached at initial presentation, presents a problem of sex assignment and should be considered a clinical emergency. It is important to identify these scenarios as early as possible and to have a care pathway that can be quickly activated. The aim of this paper is to review the process of sex assignment and areas that are contentious and to consider future directions.

Sex Development

Sex development is a process that can be broadly divided into the development of the gonads and the development of the reproductive organs and the genitalia. This process is under the control of molecular networks of male- and female-specific gene expression, dosing and interaction (2). Presence of XY chromosomes triggers activation of the SRY gene, which initiates development of a testis, where the primary sex cords develop into Sertoli cells. Sertoli cells produce anti-Müllerian Hormone (AMH) which promotes the regression of the Müllerian ducts. Leydig cells form outside the testicular tubules and produce testosterone, which stimulates the Wölffian duct to persist to form the epididymis, vas deferens and seminal vesicles. Under the influence of androgens, the genital tubercle differentiates and enlarges to become a penis, the urethral folds form the penile urethra, and labioscrotal swellings fuse to form the scrotum. In the absence of testicular development being switched on by the SRY gene on the Y chromosome, Wnt-4 signaling sustains oocyte and granulosa cell development, and suppresses Sertoli and Leydig cell differentiation. The Müllerian system of the embryo gives rise to the uterus, cervix, upper vagina, and fallopian tubes in the absence of AMH. In the absence of androgens, the phallus becomes a clitoris, the labioscrotal folds become the labia, and the urethra does not migrate to the tip of the phallus (2).

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Disorders of Sex Development

"DSD" is an umbrella term for a group of conditions that arise due to a biological variation in chromosomal, gonadal, or anatomic sex. The current classification of DSD in three subgroups, sex chromosome DSD, 46, XX DSD, and 46,XY DSD, was recommended by the international consensus group on management of intersex disorders in Chicago in 2005 (1). These disorders could be determined at different development stages of the life-cycle in fetuses or newborns with atypical external genitalia, dysgenetic gonads and internal genitalia. The term 'DSD', by itself, is not a diagnosis but a presentation characterised by a wide range of clinical features such as hypospadias (1 in 250 boys), ambiguous genitalia (1 in 4500 live births) and complete XX or XY sex reversal (1 in 20,000 births) (3,4,5). Older children and adolescents may present with clinical features such as delayed puberty, unexpected virilization or gynaecomastia, infertility, or gonadal tumors.

The first step in sexual differentiation is the activation of the SRY gene to trigger testicular development at 7-8 weeks of fetal development. When there is a mutation or deletion of SRY, or one of the early downstream genes in gonadal differentiation, then the gonads fail to mature into either ovary or testis and become nonfunctional streak gonads. Failure of testicular development leads to absent male hormones required for masculinization of both internal and external genitalia. This leads to regression of the Wölffian duct and preservation of the Müllerian duct. The external genitalia continue on the female developmental pathway, leading to a normal external female phenotype at birth. Whilst the vagina and uterus form normally in the absence of AMH, the formation of functioning ovaries requires the activation of critical ovarian development genes.

In females with non-disjunction of the sex chromosomes, leading to the 45, X genotype (Turner syndrome), the primitive germ cells are displaced from the caudal yolk-sac into the indifferent gonad. Therefore absence of the second X chromosome leads to abnormal development of the follicles. This in turn leads to premature senescence in early childhood. The germ cells undergo premature death, sometime between late foetal life and the first few years after birth. Early biopsy of the gonad, at birth or shortly afterwards, may show some primary follicles which degrade over the next few years. As a result, for patients with 45, X/46, XX mosaicism ovarian function may be occasionally sustained until later in life. In some rare forms of abnormal sex determination, there is complete sex reversal, with XY females or XX males. In the latter case, the common cause is translocation of a small segment of the Y chromosome, which includes the SRY gene, onto the X chromosome, usually at Xp11 .3. Currently this is identified by fluorescent in situ hybridisation with a marker for the SRY gene (6).

Factors That Influence Sex Assignment at Birth

The approach to sex of rearing decisions in DSD patients has changed fundamentally over time and involves many factors. Influencing factors for sex assignment include diagnosis, genital appearance, fertility potential, therapeutic and surgical options and familial views or circumstances including cultural biases. When a specific diagnosis can be reached, recommendations for sex assignment can be based upon outcome data. The assessment of the genitalia must include a description and symmetry of the external genital development including degree of virilization, Prader staging and the presence and position of gonads. Asymmetry is primarily seen as a result of greater virilization of the labioscrotal fold derived structures on one side compared with the other. This commonly results in the appearance of one side more like a labial fold and the other like a hemi-scrotum. For underdeveloped male genitalia, the capacity to respond to exogenous androgen may be a challenging method for determining sex assignment given that there are no agreed norms. Parental backgrounds and expectations, broader family dynamics, social circumstance and ethnic or cultural influences must also be considered in each case.

Temporal Trends in Attitudes

The Chicago Consensus recommended that every affected child had the right to be assigned sex and generally sex assignment is performed soon after birth. However, most health care providers allow a period where notification of birth can be delayed. In some countries such as Australia, Bangladesh, Germany, India, New Zealand, Nepal and Pakistan, the sex of the child can be registered as undetermined and the calls for this category to be more widely available internationally as well as removing sex assignment from official documents is increasing. It is possible that the need for sex in official documents such as birth certificates or passports may have been driven by the need for sex to be a distinguishing marker of identification. With increasing availability of alternative forms of biometrics, the need to have sex as a marker of identification may reduce over time. In some infants affected by DSD and especially those presenting with genital ambiguity, the issue of sex of rearing has been a debatable aspect of management. In 2006, it was stipulated that sex assignment cannot solely be based on genital appearance but should include the diagnosis, surgical options, replacement therapy, the potential for fertility, views of the family and circumstances relating to cultural approach (3). The presentation of DSD in the newborn when sex assignment is unclear has often been

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considered "a medical and social emergency". Whilst it is true that such a presentation may signify life threatening conditions such as congenital adrenal hyperplasia (CAH), a label of emergency may lead to a hastened process with inadequate communication within the team or with the family. More recently, in some countries such as Germany, parents have been given the option to delay sex assignment for longer than was previously possible and this may help with the process of sex assignment. It remains to be further studied whether these shifts in policy reduce the stigma or isolation felt by the parents or the child (7).

Recent data from the I-DSD Registry show that practice amongst specialist centres is also changing. Whereas in the past, infants with XY DSD (other than complete AIS) who had a very low external masculinization score were raised as a girl, more recently, these infants are more likely to be raised as a boy (8). Whilst this shift in practice is guided by accumulating evidence of adverse psychosocial and psychosexual outcome in those raised as girls (9), there is a continuing need to gather evidence on long-term outcome in those who are now being raised as boys. Whilst it is generally believed that 46 XX infants with CAH should be raised as girls, with the availability of long-term outcome data, some experts have questioned this practice in those infants who are severely virilised at birth, advocating that a male sex of rearing may be more appropriate (10).

Sex Assignment

The birth of a child with suspected DSD is a challenging situation for parents and health professionals (11). In many cases, a decision is made immediately after birth about the sex of the child. The possible course of future physical, emotional and sexual development of individuals with DSD must also be kept in mind, in order to make the right decision in childhood to achieve good lifelong outcomes for health, emotional and social development (12). The lack of knowledge about the relative contribution of biological (e.g., genes and prenatal sex hormone exposure) and non-biological influences (e.g., parental attitude, peer influences and cultural context) on gender development can make sex assignment more difficult. Prediction of adult gender identity is difficult in some conditions. Although there is no doubt that investigations are required in all infants with suspected DSD, there is less certainty about when investigations should be performed in those cases in which the genitalia are less ambiguous. Expert opinion suggests that groups of infants who should be evaluated include those with female genitalia with atypical features, such as an enlarged clitoris, or those with male genitalia with atypical features (13,14). Also, evaluation may be necessary in those who have a family history of DSD or there is discordance between genital appearance and a prenatal karyotype. The health care team has the important role of evaluating the patient and informing the parents about the diagnosis, possible therapies, available outcome data as well as availability of support groups (12). Surgical possibilities, potential for fertility and the need for hormone replacement should also be taken into account when necessary.

Geographical Differences

Society often plays a major role in the decision for sex assignment and the sex of rearing decision is often considered to be the parents' right, obligation and responsibility. Strong social pressures influenced by cultural, traditional and economic factors persist in some social groups, where the male may have a dominant role in financial and social life. In such communities where a man is the traditional breadwinner, choosing the male gender is often considered to be more preferable for the affected offspring than the individual's sexual potential (15,16,17). There are only a few reports about geographical differences in choosing sex of rearing. A recent study from India showed that seven infants who were 46, XX and had CAH were raised as males because of family preference, older age of diagnosis and having a "good" phallus (18). In such scenarios, the algorithm for sex assignment is over simplified and based on good or poor phallic development (19,20).

Evidence of Discontent with Assigned Sex

It is not unusual that adults with DSD experience discontent with the assigned sex. This may be attributed to several reasons including medical interventions such as surgery or hormone replacement therapy, impact of delayed or precocious development, experience of stigmatization or psychological trauma, social expectation of gender role behavior and other coexisting mental health conditions. Some studies found that girls with CAH show masculinization of behavior, such as spatial orientation, visualization, targeting, personality, cognitive abilities, and sexuality (21,22,23). Others demonstrated a masculine bias on various personality traits supporting the determining role of parental steroids in sex-role identity (e.g., Detachment and Indirect Aggression Scales, Aggression and Stress Reaction Scales, Reinish's Aggression Inventory) (24). Although women with CAH develop a female gender identity, gender dysphoria may be more common than in women without CAH (25). It was shown that five percent of adolescent and adult women with CAH suffer a form of gender dysphoria contributing to the decision for sex re-assignment. The extent of sexual activity of women with CAH may also be lower when compared with the normal population (26). A recent literature review concluded that people who were

46 XX and extremely virilized due to CAH and who were reared male may enjoy satisfactory level of social and sexual function as male adults if they obtained optimal social support (27,28). Prenatal androgen stimulation in girls with CAH results in different levels of virilization. The severity of the enzyme defect has influence on phenotype. Sexual function and the quality of sexual life in women with CAH following genital surgery with clitoroplasty and vaginoplasty has been reported in several small group studies and many report dissatisfaction with clitoral surgery (29,30,31). Medically, the low birth rate in women with CAH may be due to the influence of low gonadotropins and high progesterone levels (32).

Due to an androgen biosynthesis problem, children with 46,XY who have 5α reductase-type 2 deficiency or 17β-hydroxysteroid dehydrogenase-type 3 deficiency are usually born with female-appearing or ambiguous genitalia. In general, these infants are raised as girls and at puberty, when they start to masculinize, transition to the male role has been described (33). An increased rate of sex change from the female to the male sex role has been seen in children and adolescents with genital malformation (agenesis of the penis, cloacal exstrophy) who grow up as girls and had a normal level of male hormones at birth (34). The increase in testosterone level after puberty thus seems to be an important factor in gender identity and consolidation in individuals with these conditions. It is also possible that testosterone exposure at critical prenatal stages may have also played a role. Cultural factors should be considered, because gender role change may also occur at different rates in different societies (1).

In Complete Androgen Insensitivity syndrome (CAIS) the complete female appearance at birth usually masks the condition completely and the infants are raised without any doubt as girls. These children display typical girl behavior and female gender development, with no signs of gender dysphoria (27,34). However, it is possible that women with CAIS may be dissatisfied with their primary sex organs, even without observable gender atypical signs (35). The issue of insecurity based on their own body perception may arise due to discrepancy between gender role and karyotype. On the other hand, individuals with Partial Androgen Insensitivity syndrome (PAIS) may develop gender dysphoria (36). Approximately 25% of individuals with PAIS appear to develop gender dysphoria regardless of the sex they are reared as (37).

Many affected children with DSD undergo feminizing or masculinizing genitoplasties as well as gonadectomies. There are several reasons for these surgeries including aligning a child's phenotype more closely with their sex of rearing, determining future fertility potential, and removing the risk of malignancy (38). In those undergoing feminizing surgeries (clitoroplasty and vaginoplasty) the total excision of the clitoris is no longer recommended. The current approach is a clitoroplasty that preserves the glans and neurovascular bundle of the phallus for better genital sensation and orgasmic potential (39). The point of entry of the vagina into the urogenital sinus is important for the choice of vaginoplasty procedure. Novel methods for vaginoplasty include skin flap, sigmoid bowel, and pullthrough (36). Alternative interventions such as vaginal dilatation may also be preferred in some situations. The timing and the need for these procedures is increasingly debatable and is beyond the scope of this review on sex assignment.

Recent investigations of outcomes of gonadectomy and vaginoplasty in girls and women affected by CAIS range from satisfaction with surgery (40,41) to preference for early surgery, to a lack of sexual desire/arousal and dyspareunia attributed to these procedures (42,43). Among the factors contributing to the high dissatisfaction with treatment in this subgroup are the lack of information provided to the patient about their condition and its management so that they can make an informed decision for themselves. It is unclear if improved surgical techniques have resulted in higher patient satisfaction, since age did not influence the satisfaction rates with surgery (42). On the other hand, women with 46,XY DSD without genitoplasty and born with female external genitalia were mostly satisfied with their vaginal length and clitoral arousal (44). However, a recent Dutch study with a mix of people with XY DSD and CAH reported impairment on the female sexual function index and were at risk of developing sexual dysfunction, non-operated patients with CAIS and complete gonadal dysgenesis were significantly more dissatisfied with sexual life than operated women with XY DSD or CAH. This study showed that a large proportion of women reported problems of coping with diagnosis, distress of infertility and suffering from societal ignorance (43,44). It is therefore possible that these are the major contributory factors in the impairment of psychosexual and psychosocial life in XY DSD.

Masculinizing surgeries for DSD include release of ventral chordee, hypospadias repair, gonadectomies and placement of prosthetic testes in the scrotum at puberty. Many studies have found that men with hypospadias repair in childhood still report at least some degree of dissatisfaction with their genital appearance and size, which may lead to psychosexual distress and jeopardize sexual well-being (45). In 46 XY DSD with micropenis, it is not only the genital appearance, but also overall physical development - such

as male development and eventual breast growth - as is the case in PAIS - that can lead to a negative body image and impaired social interactions (46). Retrospectively, it is increasingly clear that masculinizing genitoplasty in severe cases of hypospadias may require many more procedures than feminizing genitoplasty and may also result in a poorer cosmetic outcome (46). In comparison to those who develop a male gender, patients with 46,XY DSD reared male who ultimately develop a female gender do not experience different cosmetic or functional outcomes from their genitoplasty (44). Postoperative complications (fistulae, urethral strictures and meatal stenosis and repeated surgical procedures are of particular prospective concern because of associated scarring and loss of tissue, as well as the estimated negative impact on sexual function (47). In addition, penile lengthening procedures, such as in hypospadias repair in men with penile deficiency, can only elongate the penis by an average of 1.5-2.5 cm. Whether or not a correlation exists between small penile length and dysfunctional penetrative intercourse remain unclear, although a penile length of more than 6-7 cm seems to constitute a premise for successful sexual contact (48,49,50). Some authors described a few men with micropenis who reported a mutually satisfying sex life with their heterosexual partners (51,52).

Summary

Differences in DSD management will result from a combination of traditional beliefs, folk remedies and prejudices, fed by rumour and discrimination and available healthcare resources and expertise (18). Nevertheless, it should be appreciated that people with DSD have the same desires as everyone else: to find a peer who will love them; to be a valuable part of society; to be comfortable with their body; to be able to have satisfactory sexual relations; to integrate into the community; and to trust their medical caregivers. More clinical studies as well as academic and public debate are needed to support people with DSD with sex assignment, gender identity development, atypical gender role behavior, sexual orientation and satisfaction with their own sexuality. It is debatable whether the dissatisfaction that people with DSD experience with the allocated sex of rearing is a gender identity disorder or not. People with DSD who are discontent may simply be showing an evolving discrepancy between the gender identity they experience and the sex of rearing which, in most cases was chosen by their carers at birth. All carers, parents and professionals, should be aware that possibility of dissatisfaction with the assigned sex, however small, does exist and centres that provide expert care should be prepared to support the patient and the family, if required.

Finally, healthcare workers should share expertise and collaborate globally in prospective studies as it is essential to gain insight into the outcome of individuals affected by these rare conditions. The variations in practice can be decreased through networks of clinical and research centers. Disease registries are playing a significant role in development and improvement of networks. Establishment of the DSD registry in 2007, initially as the ESPE DSD Registry, followed by the Euro-DSD Registry and currently as the I-DSD Registry, is a perfect example of how registries can evolve and also be used to address issues ranging from fundamental mechanisms to clinical practice and health care outcomes (8). It is likely that newly established international collaborations to generate sufficient numbers for the study of very rare disorders will provide better information on which new protocols can be developed.

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Literature Search: Renata Markosyan, S. Faisal Ahmed, Writing: Renata Markosyan, S. Faisal Ahmed.

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REVIEW

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Update on the Genetics of Idiopathic Hypogonadotropic Hypogonadism

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Abstract

Traditionally, idiopathic hypogonadotropic hypogonadism (IHH) is divided into two major categories: Kallmann syndrome (KS) and normosmic IHH (nIHH). To date, inactivating variants in more than 50 genes have been reported to cause IHH. These mutations are estimated to account for up to 50% of all apparently hereditary cases. Identification of further causative gene mutations is expected to be more feasible with the increasing use of whole exome/genome sequencing. Presence of more than one IHH-associated mutant gene in a given patient/pedigree (oligogenic inheritance) is seen in 10-20% of all IHH cases. It is now well established that about 10-20% of IHH cases recover from IHH either spontaneously or after receiving some sex steroid replacement therapy. Moreover, there may be an overlap or transition between constitutional delay in growth and puberty (CDGP) and IHH. It has been increasingly observed that oligogenic inheritance and clinical recovery complicates the phenotype/genotype relationship in IHH, thus making it challenging to find new IHH-associated genes. In a clinical sense, recognizing those IHH genes and associated phenotypes may improve our diagnostic capabilities by enabling us to prioritize the screening of particular gene(s) such as synkinesia (*ANOS1*), dental agenesis (*FGF8/FGFR1*) and hearing loss (*CHD7*). Also, IHH-associated gene studies may be translated into new therapies such as for polycystic ovary syndrome. In a scientific sense, the most significant contribution of IHH-associated gene studies has been the characterization of the long-sought gonadotropin releasing hormone pulse generator. It appears that genetic studies of IHH will continue to advance our knowledge in both the biological and clinical domains.
Keywords: Hypogonadism, hypogonadotropic, delayed puberty, genetics, etiology

Introduction

The activity level of the hypothalamo-pituitary-gonadal (HPG) axis is remarkably variable throughout life. A gradual increase of HPG activity around the beginning of the second decade of life brings about sex-specific, secondary sexual features and a maturing reproductive system. This specialized phase of human development is called puberty and lasts from two to five years. Absence of puberty manifests itself as sexual immaturity and reproductive incompetence, which can be succinctly termed as hypogonadism. If lack of such development is due to anatomical or functional defects, resulting in reduced gonadotropin releasing hormone (GnRH) and/or gonadotropin release, the condition is called hypogonadotropic hypogonadism (HH).

1. Idiopathic Hypogonadotropic Hypogonadism

The term idiopathic HH (IHH) is used to define those IHH cases with no apparent causes. Traditionally, IHH is divided into two major categories: Kallmann syndrome (KS) and normosmic IHH (nIHH). IHH can be congenital or acquired. The great majority of hereditary causes of IHH are congenital. Typically, in girls there is no clinical manifestation of IHH before the early teen years. In boys, since the HPG axis is very active roughly between the 16th and 22nd week of gestation and androgenic end products of this period are required for normal virilization of the 46,XY fetus, male infants with IHH may have micropenis and/or cryptorchidism at birth. Under-virilization of the male can be severe enough to call for an evaluation of a "disorder of sexual development". A slight and temporary reactivation of

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the HPG axis in early infancy (around four to sixteen weeks) is called "minipuberty" and provides a unique opportunity to diagnose both male and female infants with congenital IHH (1).

KS is often due to the embryonic maldevelopment and/or interrupted migration of GnRH specific neurons. Since the embryonic migration of GnRH neurons from the nasal placode towards their final destination in the hypothalamus occurs in association with olfactory receptor neurons, the resulting phenotype includes anosmia in addition to HH. KS cases often have additional congenital anomalies such as cleft palate, unilateral renal agenesis, split hands and feet, short metacarpals, deafness, and mirror movements (synkinesia).

In contrast nIHH refers to those IHH cases not associated with anosmia (2). nIHH results from the dysfunction of the normally sited GnRH neurons in the hypothalamus. These cases typically do not have any accompanying congenital lesions.

However, one should be careful when using these terms because the line between KS and nIHH is sometimes blurred, as most typically seen with *FGFR1* mutations. Furthermore, there may be pathophysiological overlaps between the two entities. For example, patients with *CCDC141* or *IGSF10* mutations have nIHH despite showing *in vitro* evidence of impaired migration of the GnRH neurons (3,4).

Pubertal delay is the most typical presentation of IHH. Pubertal delay is defined as absence of breast development (Tanner breast stage 1) in a girl at age 13 or failure to achieve a testicular volume of 4 mL in a boy by age 14 (5). By far the most common cause of delayed puberty is constitutional delay in growth and puberty (CDGP), which is not a disease *per se* but a maturational delay in development at the extreme of the population standards. CDPG accounts for pubertal delay in two third of boys and one third of girls (6). CDGP is a diagnosis of exclusion and should often be considered in the differential diagnosis of IHH. To distinguish between these two conditions often requires lengthy workup and observation periods.

It has been shown that some variants in known puberty genes such as *TAC3* and *TACR3* are shared by individuals with IHH or CDGP within the same family, suggesting that CDGP shares an underlying pathophysiology with IHH, only representing a milder form of the same genetic dysfunction (7). Clinicians often successfully try a low dose sex steroid course to "jump start" pubertal development in patients with suspected CDGP. It is now well established that about 10-20 % of IHH cases recover either spontaneously or more typically after receiving some sex steroid replacement therapy (8,9). These foregoing observations further suggest that CDGP and IHH may have common pathophysiological underpinnings. Therefore, it appears that there is a continuum of phenotype from normal timing of pubertal development all the way to extreme IHH, encompassing CDGP along the way.

2. Genes Associated with Idiopathic Hypogonadotropic Hypogonadism

Currently known genetic defects account for up to 50 % of all IHH cases (10). To date mutations in around 50 genes have been reported to cause IHH. The full current list of genes associated with IHH is shown in Table 1. Presence of more than one IHH-associated mutant gene in a patient/pedigree (oligogenic inheritance) is thought to account for 10-20 %

Table 1. Genetic causes of idiopathic hypogonadotropic hypogonadism

Category	Mutated genes
Disorders of the embryonic migration of the GnRH neuron (Kallmann syndrome)	*ANOS1 (KAL1), FGFR1, FGF8, FGF17, IL17RD, DUSP6, SPRY4, FLRT3, KLB, PROK2, PROKR2, HS6ST1, CHD7, WDR11, SEMA3A, SEMA3E, IGSF10, SMCHD1, CCDC141, FEZF1*
Disorders of the GnRH pulse generator	*TAC3, TACR3, KISS1, KISS1R, GNRH1*
Developmental disorders of Hypothalamic-pituitary region	*NR0B1 (DAX1), NR5A1, SRA1, HESX-1, LHX3, PROP-1, SOX2*
Disorders of the pituitary gonadotropes	*GNRHR, FSHB, LHB*
Disorders of IHH associated with obesity	*LEP, LEPR, PC1*
Disorders of IHH associated with neurodegenerative syndromes	**Gordon Holmes syndrome:** Cerebellar ataxia +/- retinal dystrophy *(PNPLA6, RNF216, OTUD4, STUB1)*
	4H syndrome: Hypomyelination, hypodontia *(POLR3A, POLR3B)*
	Warburg Micro syndrome/Martsolf syndrome: microcephaly, microcornea, mental retardation, optic atrophy *(RAB3GAP1, RAB3GAP2, RAB18, TBC1D20)*
	DMXL2: non-autoimmune insulin deficiency diabetes mellitus, hypoglycemia, central hypothyroidism, mental retardation, and peripheral demyelinating sensorimotor polyneuropathy

of all IHH cases (11,12,13,14). With the increasing use of unbiased comprehensive genetic studies such as whole exome sequencing (WES), it is now known that oligogenic inheritance is more common than previously thought in various Mendelian disorders (15).

2a. Kallmann Syndrome Associated Genes

X-linked recessive, autosomal dominant (AD) and autosomal recessive (AR) patterns of inheritance have been reported. However, KS is often sporadic; even if it is familial, a substantial variability in clinical phenotype of the same genetic defect among affected family members may be seen (16,17,18). According to the presence of certain associated clinical features, genetic screening for particular gene(s) may be prioritized: synkinesia (KAL1), dental agenesis (FGF8/FGFR1), digital bony abnormalities (FGF8/FGFR1) and hearing loss (*CHD7, SOX10*) (19). As a common pathophysiological denominator with KS genes, fibroblast growth factor signaling, prokineticin signaling and Anosmin-1 appear to interact with heparin sulfate glycosominoglycan compounds within an extracellular signaling complex to promote GnRH neuronal migration (20,21).

ANOS1 (KAL1)

The *ANOS1* gene, encoding an extracellular glycoprotein called Anosmin-1, associates with the cell membrane *via* heparin sulphate proteoglycans (HSPG) (22). Ten to twenty percent of males with KS carry *KAL1* mutations or intragenic microdeletions are present (23,24). Most pathogenic mutations entirely disrupt protein function. The inheritance pattern is X-linked recessive. The KS phenotype produced by *ANOS1* mutations seem not only more severe but also less variable than that seen with other known molecular defects (24,25). Accompanying clinical features include synkinesia and unilateral renal agenesis, which occurs in 75% and 30% of patients respectively (26).

FGFR1, *FGF8* and Related Genes (*FGF17, IL17RD, DUSP6, SPRY4, FLRT3*, and *KLB*) (20,27,28)

FGFR1 requires both HSPG as a co-receptor and Anosmin-1, which is also HSPG-associated. Anosmin-1 is likely to play a role in mediating FGFR1 signaling (21). Loss of *FGFR1* function has been reported to elicit reproductive abnormalities ranging from severe AD KS through fully penetrant nIHH to delayed puberty (29,30,31,32,33). Around 10% of patients with KS were found to have inactivating mutations in *FGFR1* (20,29,30). More recently, loss-of-function mutations in *FGFR1* were detected in 7% of 134 nIHH patients, suggesting that FGFR1 should be one of the major genes in screening panels for nIHH patients (34).

In 2008, *FGF8*, one of 11 ligands of FGF signaling was found to be mutated in six out of 461 (1.5%) IHH patients. These patients exhibited varying levels of olfactory function and HH (27). Furthermore, mice homozygous for the hypomorphic *FGF8* allele exhibited absent olfactory bulbs and lacked GnRH neurons in the hypothalamus (27). As for the features of *FGF8/FGFR1* loss of function, cleft palate is found in up to 30% of patients, while cartilage abnormalities in either ear or nose and some digital anomalies have been reported (26). Further screening for FGF8 related genes in a group of 388 congenital IHH patients revealed inactivating variants in *FGF17, IL17RD, DUSP6, SPRY4,* and *FLRT3* (28).

KLB

KLB is the most recently reported Fibroblast growth factor related IHH gene (35). *KLB* encodes for Beta-Klotho, which is a co-receptor in FGF21 signaling through the FGFR1 product. The authors of this paper screened more than 300 IHH patients and found 13 patients with loss of function mutations. They also reported that the majority of patients with KLB mutations exhibited some degree of metabolic defect such as insulin resistance or dyslipidemia. The *KLB* knock out mouse model revealed a milder hypogonadal phenotype when compared to the corresponding human phenotype (35).

PROKR2 and *PROK2*

The *PROK2* gene encodes prokinetecin 2, an 81 amino acid peptide that signals *via* the G protein-coupled product of the *PROKR2* gene. This ligand and its receptor were recognized as strong candidates for KS as *PROK2* (36,37) or *PROKR2* knockout mice had defective olfactory bulbs and failed migration of GnRH neurons (38). Subsequently, inactivating variants in *PROKR2* or *PROK2* were detected in KS patients. Most of these mutations were heterozygous, although both homozygous and compound heterozygous mutations have been described (39). Patients with *PROK2* or *PROKR2* mutations have considerable phenotypic variability (37,40,41), ranging from KS to nIHH. A variety of accompanying clinical features including fibrous dysplasia, synkinesia and epilepsy have been reported in patients with *PROK2* or *PROKR2* mutations. It appears that mutations in *PROKR2* and *PROK2* are often found in combination with other mutations in IHH with oligogenic inheritance.

CHD7

The *CHD7* gene encodes a chromatin-remodeling factor and is mutant in CHARGE syndrome, which has the constellation of Colobomata, Heart Anomalies, choanal Atresia, Retardation, Genital and Ear anomalies (42). Some patients also have IHH and hyposmia. Based on the hypothesis that KS and nIHH may be a milder allelic variant

of CHARGE syndrome, *CHD7* was screened in 197 patients with KS or nIHH but devoid of CHARGE features. Mutations were identified in three KS and four nIHH patients (43). In another study, three of 56 KS/nIHH patients had mutations in *CHD7* (44). The authors suggest that patients diagnosed with KS should be screened for clinical features consistent with CHARGE syndrome. If such features are present, particularly deafness, anomalous ears, coloboma and/or hypoplasia or aplasia of the semicircular canals, CHD7 should be tested (44).

WDR11

The *WDR11* gene product partners EMX1, a homeodomain transcription factor involved in the development of olfactory neurons. By positional cloning, heterozygous mutations were discovered in several patients with KS (45). Recently, a digenic combination of monoallelic variants in *PROKR2* and *WDR11* has been reported to be responsible for a pituitary stalk interruption syndrome in a child (46).

SEMA3A

SEMA3A encodes for semaphorin 3A, a protein that interacts with neuropilins. Mice lacking semaphorin 3A expression have been demonstrated to have a Kallmann-like phenotype. Screening large groups of patients with KS revealed a variety of monoallelic mutations. Some of these mutations coexist with other KS causing gene mutations, further showing oligogenic inheritance in IHH (47,48). In a recent study in patients with IHH, heterozygous missense variants in SEMA3A and SEMA7A were found in association with second variants in other IHH genes (49).

SEMA3E

Semaphorin 3E (*SEMA3E*) is a secreted protein that modulates axonal growth. A *SEMA3E* missense mutation was recently reported in two brothers with KS (50). Functional studies have shown that *SEMA3E* may act as a survival factor for maturing hypothalamic GnRH neurons.

SOX10

Inactivating mutations in *SOX10* cause Waardenburg syndrome, a rare disorder characterized by pigmentation abnormalities and hearing impairment. Screening for *SOX10* mutations in KS patients with deafness revealed inactivating variants in approximately one-third of them. *SOX10* knockout mice showed absence of olfactory ensheathing cells along the olfactory nerve pathway (51).

HS6ST1

HS 6-O-sulfotransferase 1 is a sulfation enzyme that specifically and non-randomly modifies heparan sulfate, an important extracellular matrix component, which is probably required for optimal cell-cell communication, such as during olfactory neuronal migration and ligand-receptor interactions. Recently, inactivating *HS6ST1* mutations, in association with other KS gene mutations, have been reported in seven families with KS (52).

CCDC141

CCDC141 encodes a coiled-coil domain containing protein that is expressed in GnRH neurons. We have reported inactivating *CCDC141* variants in four separate families with IHH. Affected individuals had normal olfactory function and anatomically normal olfactory bulbs (53). In a rodent nasal explant model, knockdown of *CCDC141* resulted in decreased embryonic GnRH cell migration without interrupting olfactory axon outgrowth (3).

FEZF1

FEZF1 encodes a transcriptional repressor that is expressed during embryogenesis in the olfactory epithelium, amygdala and hypothalamus. The *FEZF1* gene product promotes the presence of a protease to enable olfactory receptor neurons, and thus accompanying GnRH neurons, to enter the brain (54). Recently, using autozygosity mapping and WES in a cohort of 30 individuals with KS, we identified homozygous, loss-of-function mutations in *FEZF1* in two independent consanguineous families, each with two affected siblings (55).

IGSF10

IGSF10 is a member of the immunoglobulin superfamily. Howard et al (4) obtained WES data on more than 100 individuals with delayed puberty and identified IGSF10 mutations in six families. The knock down studies revealed reduced GnRH migration in the GN11 cell line. Despite having impaired migration of GnRH neurons, the patients carrying these mutations had a normal sense of smell. The authors suggested that reduced number or delayed arrival of neurons in the hypothalamus leads to a somewhat milder functional defect in the formation of the GnRH neuronal network with eventual delayed puberty but not permanent IHH. Interestingly, they also identified mutations in adult individuals with functional hypothalamic amenorrhea, which is considered a form of mild, transient HH (4).

SMCHD1

SMCHD1 encodes for an epigenetic repressor which is expressed in the human olfactory epithelium. Shaw et al (56) demonstrated inactivating *SMCHD1* mutations as the cause of congenital absence of nose in 41 cases. The great majority of patients (97%) also had hypogonadal features such as cryptorchidism, microphallus or amenorrhea, along with absent olfactory structures and anosmia.

2b. Normosmic Idiopathic Hypogonadotropic Hypogonadism (nIHH) Associated Genes

nIHH-causing genes are more pertinent to the understanding of the function of the HPG axis and puberty. Identified mutations in familial cases of nIHH has led to greater understanding of this function. In a study on 22 consecutive, multiplex families with nIHH, we identified mutations in five genes (*GNRHR, TACR3, TAC3, KISS1R*, and *KISS1*) in 77% of them. *GNRHR* and *TACR3* mutations were the two most common causative mutations, occurring with about equal frequency in two third of the mutation identified cases (57).

LEP and *LEPR*

Leptin deficiency with mutations in either encoding leptin (*LEP*) or encoding the leptin receptor (*LEPR*) is associated with IHH (58,59). The administration of leptin in LEP-deficient patients restores normal pubertal development but does not cause early puberty in prepubertal children, which implies that leptin is a permissive factor for the development of puberty in humans (60). These patients are easily recognizable among other IHH patients with because of the presence of early onset obesity and hyperphagia.

NR0B1 (DAX1)

NR0B1 is an orphan member of the nuclear receptor superfamily. Inactivating variants in the *NR0B1* gene cause X-linked congenital adrenal hypoplasia with HH (61). Adrenal hypoplasia typically presents as adrenal insufficiency during infancy, whereas HH becomes manifest in affected males who survive into the second decade of life.

SRA1

SRA1 was the first gene shown to function through both its protein and noncoding, functional RNA products (62). These products act as co-regulators of nuclear receptors, including sex steroid receptors as well as SF-1 and LRH-1, the master regulators of steroidogenesis. *SRA1* is required for the synergistic enhancement of SF-1 transcriptional activity by *DAX-1 (NR0B1*), mutations in which also cause IHH, as discussed above (63). WES and autozygosity mapping studies revealed three independent families in which IHH was associated with inactivating *SRA1* variants (64).

GNRHR and *GNRH1*

GNRH1 and *GNRHR* are the most obvious candidate gene in the etiology of IHH. *GNRHR* defects produce AR, isolated nIHH, with no evidence of accompanying developmental defects such as hyposmia (65,66,67). GNRHR mutations have been suggested to account for about 40-50% of familial AR nIHH, and around 17% of sporadic nIHH (66). In a recent survey of 110 patients with nIHH, eleven IHH patients (10%) carried biallelic *GNRHR* mutations while none of the 50 patients studied with CDGP had any deleterious variants (68). To date, more than 25 different mutations have been reported. Interestingly, only seven years ago the first inactivating homozygous mutations in *GNRH1* itself causing IHH were reported by two independent groups (69,70). In these cases IHH was shown to be reverseable by pulsatile GnRH administration, confirming the pivotal role of GnRH in human reproduction (69). Out of 310 patients with IHH, only one case was found, attesting to the rarity of mutations in this gene as a cause of IHH (70). We recently reported further *GNRH1* mutations located in the region encoding the decapeptide which is the same region involved in earlier reported mutations (71).

KISS1R and *KISS1*

KISS1R (formerly *GPR54*) encodes for the receptor for small peptides derived from the *KISS1* gene and it was previously thought not to play a role in the HPG axis (72). Mutations in *KISS1R* were first reported in IHH familial multiplex cases in 2003 (73,74). Ensuing studies established kisspeptin signaling as an essential, positive regulator of GNRH secretion. In a mutational screening study, only five out of 166 (3%) probands with nIHH were found to have rare variants in *KISS1R* (75). Studying a large, consanguineous family with four sisters with nIHH, we found inactivating mutations altering the 4th amino acid of Kisspeptin-10. Overnight frequent LH sampling did not reveal any LH pulsatility, further confirming the essential role of kisspeptin signaling in the GnRH pulse generator (76).

TACR3 and *TAC3*

Tachykinin receptor-3 encoded by *TACR3* is the mediator of biologic actions of neurokinin B (NKB) encoded by *TAC3*. In an effort to identify novel genes playing a role in driving the HPG axis, based on autozygosity mapping (77), we identified homozygous non-synonymous mutations in the coding sequences of *TAC3* or *TACR3* in nine patients from four families with an nIHH phenotype (78). With the additional cases identified in our cohort, it became clear that *TACR3* mutations are almost as common as *GNRHR* mutations (57). Other groups have made similar observations concerning the prevalence of *TACR3* mutations. Gianetti et al (79) found 19 among 345 (5.5%) cases while a very similar rate (5.2%) was observed by Francou et al (80) from a cohort of 173 cases of familial and sporadic nIHH. The frequent presence of a micropenis and cryptorchidism in mutant *TACR3* male patients indicates that intact *TACR3* function is also required for normal fetal gonadotropin secretion, which stimulates testicular size and descent and penile growth (1).

Clinical reversibility, evident by spontaneous progression of puberty, often following a period of exogenous sex steroid

treatment, was seen in 10% of an unselected nIHH cohort (8). A much greater percentage of reversibility (83%) was reported by Gianetti et al (79) in their *TAC3/TACR3* cohort 2010 (79). In our cohort four patients from three independent and ethnically different families showed clinical recovery among 16 (25%) patients. Interestingly, all of these families harbored the same *TACR3* mutation (p.T177K). Our studies are ongoing in an attempt to gain insight into the clinical recoverability and/or reversibility of this variant. With such a high rate of reversibility, a legitimate question arose as to whether CDGP was a form of IHH caused by *TACR3* mutations. To answer this question, Vaaralahti et al (81) screened these genes in 146 Finnish subjects with CDGP and found no variants to account for this phenotype.

Other clinical studies have provided additional valuable insight in to the biology of the HPG axis. Young et al (82) were able to produce pubertal levels of gonadotropin and sex steroids with repeated administration of GnRH in patients with Null mutations in *TAC3*, indicating that the site of NKB action is proximal to GnRH and the pituitary (82).

3. Scientific Significance of Identifying IHH-Associated Genes

Undoubtedly, the most significant contribution of IHH-associated gene studies has been the characterization of the long sought-after GnRH pulse generator. A surge of studies over the past ten years on Kisspeptin and NKB signaling, following the identifications of their inactivating mutations among familial patients with nIHH, has led to characterization of the GnRH pulse generator. According to the current understanding there is a network of sex-steroid responsive neurons in the arcuate (infindubular) nucleus that coexpress Kisspeptin, NKB, Dynorphin and ERα (KNDy or Kisspeptin neurons). Within these cells, the stimulatory NKB starts an action potential that is suppressed by the inhibitory Dynorphin. When the inhibitory effect of Dynorphin is overcome another stimulatory NKB action takes over. The net result is continuous, intermittent action potentials. Each action potential translates into a pulsatile secretion of Kisspeptin on to the axons of the GnRH neurons in the median eminence, thence GnRH is released towards the pituitary gonadotropes, via the portal circulation. Synchronization of KNDy cells is believed to be provided by NKB-NK3R signaling through ipsi- and contralateral projections among these cells (83,84,85).

4. Clinical Significance of Identifying IHH-Associated Genes

IHH-associated gene studies have provided clues for targetting diagnostic molecular genetic studies. *GNRHR* and *TACR3* should be the first two genes to be screened for diagnostic purposes in a clinical setting for equivocal cases, such as constitutional delay in puberty vs. IHH. In KS, according to the presence of certain accompanying clinical features, genetic screening for particular gene(s) may be prioritized, for example if the patient has synkinesia then *KAL1* would be suggested, dental agenesis is associated with *FGF8/FGFR1*, digital bony abnormalities also with *FGF8/FGFR1* and hearing loss with *CHD7* and *SOX10*.

IHH-associated gene studies may be translated into new therapeutic modalities. For instance, an antagonist of the *TACR3* gene product has been in clinical trial for polycystic ovarian syndrome (86).

5. Concluding Remarks

Currently, around half of the IHH genes remain to be identified. Complicated genotype/phenotype relationships in IHH, due to two well-established phenomena, oligogenic inheritance and spontaneous or induced clinical reversibility, make identifying these unknown genes challenging. Nonetheless, with the help of contemporary sequencing technologies, it appears that studies into the genetics of hypogonadotropic hypogonadism will continue to advance our knowledge in both the biological and clinical domains.

Ethics

Peer-review: Internally peer-reviewed.

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