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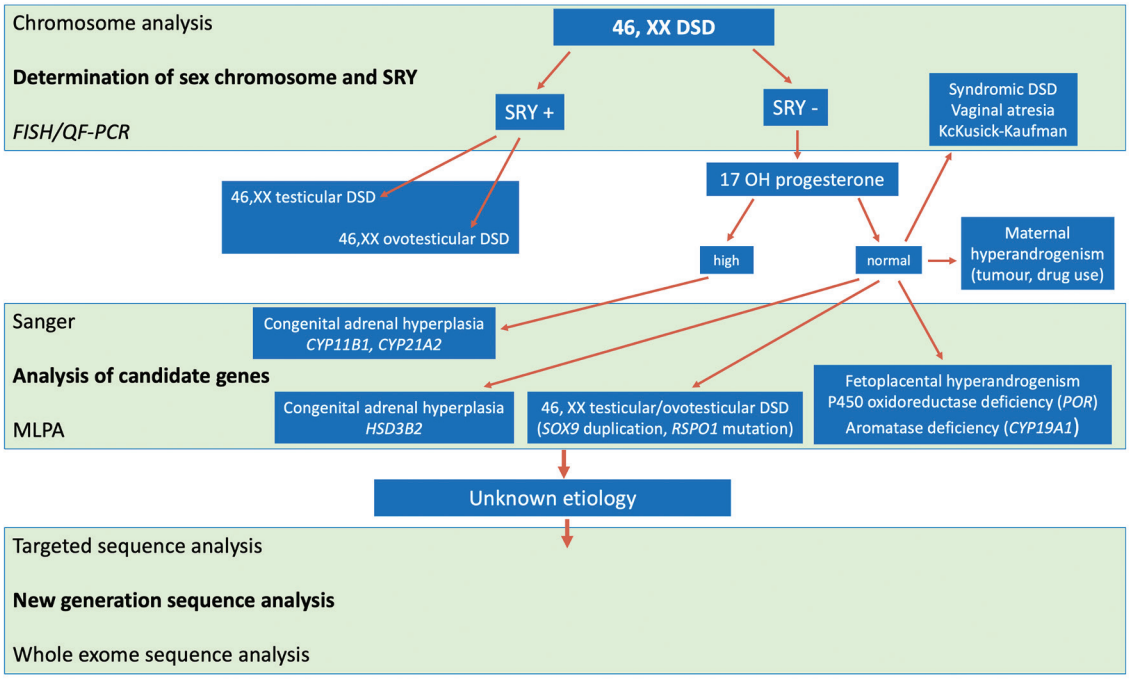

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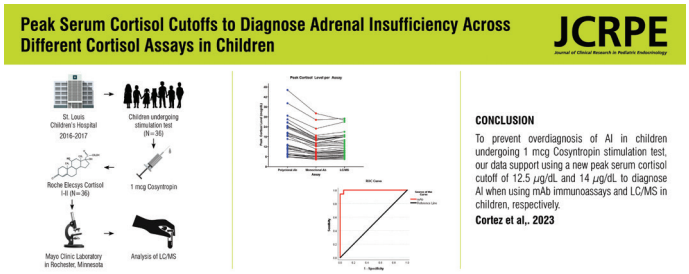

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REVIEW

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A Current Perspective on Delayed Puberty and Its Management

Ayhan Abacı, Özge Besci

Dokuz Eylül University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Turkey

Abstract

Delayed puberty is defined as the lack of development of secondary sex characteristics in childhood. Based on a review of the literature, delayed puberty can be divided into three main categories: (i) hypergonadotropic hypogonadism (congenital and acquired); (ii) permanent hypogonadotropic hypogonadism (congenital and acquired); and (iii) transient hypogonadotropic hypogonadism [constitutional delay of growth and puberty (CDGP) and functional hypogonadotropic hypogonadism]. CDGP is the most common cause of hypogonadism in both males and females, accounting for 60% and 30% respectively. Testosterone is the primary treatment for male hypogonadism, while estrogen and progesterone are used for female hypogonadism. However, in recent years, physiological induction therapy protocols such as human chorionic gonadotropin (hCG) monotherapy, hCG + follicle-stimulating hormone combined therapy, and gonadotropin-releasing hormone infusion have been recommended for the treatment of hypogonadotropic hypogonadism to increase long-term fertility success. There is no clear consensus on treatment protocols for physiological induction treatment and its effect on fertility. This review will discuss the clinical approach to hypogonadism, as well as traditional and physiological induction protocols.

Keywords: Hypogonadism, classification, treatment

Introduction

Puberty is a period of transition and developments resulting from a series of events starting *in utero* that are coordinated by the complex and timely interactions between the hypothalamus, pituitary gland, and gonads (1,2,3). In the normal tempo of maturation, in healthy infants, gonadotropin-releasing hormone (GnRH) secreting neurons in the hypothalamus, originating from the olfactory placode and the neural crest, start secreting GnRHs during the first six months of life in boys and two years in girls (4,5). During this period, also known as "mini-puberty", the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) reach pubertal ranges between 1-3 months of life and decrease to prepubertal levels around six months in boys, while they can remain elevated for up to 3-4 years in girls (6).

In boys, LH induces the maturation of Leydig cells, which secrete testosterone and insulin-like peptide 3 (INSL3), both of which are responsible for inguinoscrotal testicular descent and penile growth (7). Increased FSH levels stimulate Sertoli and germ cell proliferation, which constitute 90% of testicular volume (7). Androgens are of major importance in the onset of spermatogenesis. However, spermatogenesis is not observed during the mini-puberty period due to very low androgen receptor (AR) expression in Sertoli cells during the first year of life (as experimentally confirmed in mice) (7,8). In girls, LH induces ovarian follicular theca cells to secrete androgens, and in granulosa cells under FSH stimulation, they are aromatized to estrogens (3).

The development of pubic hair (pubarche) is not usually considered a sign of pubertal onset because pubarche can result from the maturation of the adrenal glands (adrenarche), and the appearance of pubic hair can be independent of activation of the hypothalamic-pituitary-gonadal (HPG) axis. Adrenarche is the maturation of the zona reticularis of the adrenal gland, resulting in increased production of adrenal androgens. These androgens are associated with secondary

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Address for Correspondence: Ayhan Abacı MD, Dokuz Eylül University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Turkey
E-mail: ayhanabaci@gmail.com **ORCID:** orcid.org/0000-0002-1812-0321

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sexual characteristics, such as the development of pubic and axillary hair, body odor, and acne (9).

After this transitory period, GnRHs are inactivated until they are reset again at the onset of puberty, which is influenced by several factors, including genetic differences, exercise, nutrition, endocrine-disrupting chemicals, psychosocial factors, and body mass (9,10). Physiologically, starting between the ages of 8 and 13 in girls and 9 and 14 in boys, GnRH neurons start pulsatile GnRH secretion, activating FSH and LH, which in turn further stimulate the production of sex steroids (10,11). Pubertal sex steroids induce the development of secondary sexual characteristics and fertility, starting with breast development and uterine growth in girls and testicular and penile growth in boys (12). Sex steroids in puberty also influence the accrual of bone mineral density, changes in body composition and height, metabolic responses, and general well-being in both sexes (3,13). Any damage to this complex network can cause hypogonadism in both males and females (2,12).

Definition of Delayed Puberty

Delayed puberty is defined as lack of the initial signs of sexual maturation by an age that is more than 2-2.5 standard deviation above the mean for the population (1,12,14). Delayed pubertal onset is considered in the absence of testicular enlargement (testicular volumes < 4 mL) by the age of 14 years in boys and breast development (absence of glandular breast tissue) by the age of 13 years in girls. Even if the onset is within the normal ranges, delayed pubertal progression or pubertal arrest is considered when the period between the onset and completion of puberty is longer than five years in boys, or there is a lack of menarche by 15 years of age, or within three years of thelarche in girls (3,15).

Classification of Delayed Puberty

There is no clear consensus in the literature on the classification of delayed puberty. Based on a review of the literature and text books, delayed puberty can be divided into three main categories (Table 1) (9,15,16,17,18): (i) hypergonadotropic hypogonadism (congenital and acquired); (ii) permanent hypogonadotropic hypogonadism (congenital and acquired); and (iii) Transient hypogonadotropic hypogonadism [constitutional delay of growth and puberty (CDGP) and functional hypogonadotropic hypogonadism (FHH)] (Table 1) (1,15,17,19). The etiological distribution of delayed puberty in the study by Sedlmeyer and Palmert (20) (n = 232, 158 males) is summarized in Figure 1.

Hyper- and Hypogonadotropic Hypogonadism

Hyper- and hypogonadotropic hypogonadism can be congenital (permanent or transient) or acquired (3).

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism, characterized by low gonadotropins, can be caused by either a permanent (isolated or in combination with other pituitary hormone deficiencies) or a transient deficiency caused by either a primary delay in HPG axis maturation (known as CDGP) or a secondary delay in HPG maturation (known as FHH) (15,19).

Congenital Hypogonadotropic Hypogonadism

There has been no definitive epidemiological investigation of the prevalence of congenital hypogonadotropic hypogonadism (CHH). There is a scarcity of estimates for the incidence of CHH and Kallmann syndrome (KS). Studies based on French and Sardinian military screening suggest varied incidences, with CHH occurring in 1 in 10,000 males and KS occurring in 1 in 84,000 males. In the Finnish population, the incidence of KS is estimated to be 1 in 30,000 for males and 1 in 125,000 for females (21,22,23). Male patients experience it two to five times more frequently than female patients. CHH may be sporadic or familial (9). An increasing number of genetic loci involved in either the development and migration of GnRH neurons or the secretion and action of GnRH have been implicated in CHH. More than 30 genes have been identified for isolated or multiple anterior pituitary hormone deficiency associated with CHH (24). There are several known mechanisms of transmission, including autosomal dominant transmission, X-linked recessive transmission, autosomal recessive transmission, and transmission connected to an imprinting locus (3).

It is also worth noting that isolated CHH is a complex entity. Approximately half of patients with CHH exhibit a condition called KS, which is characterized by an impaired sense of smell. The other half have been reported to have normosmic (normal sense of smell) HH (15). There is a wide spectrum of phenotypes due to multiple causes and incomplete penetrance. KS has been associated with mutations in genes, including *ANOS1, SEMA3A,* and *TUBB3*, and normosmic types have been reported to be caused by mutations in *GNRH1, KISS1, TAC3,* and *NR0B1* (25). Some of these mutations (*FGF1, PROK2, PROKR2, GNRHR*) can also result in partial loss-of-function, leading to partial hypogonadism, which is characterized by arrest of pubertal development, and even reversible HH with relatively low gonadotropin levels (3,9,26).

KS can be sporadic or familial (autosomal dominant, recessive, X-linked, digenic, and oligogenic inheritance patterns) and present with diverse phenotypical features (21). During the neonatal period, the identification of

micropenis and undescended testes (5-40%) in males are important physical examination findings in CHH. Conversely, there is no particular finding that relates to females. Male patients with partial hypogonadism may have no clinical findings in the neonatal period (8,9). The presence of severe hypospadias excludes the diagnosis of CHH (2,12,27). Absent virilization or low libido in males and the absence of breast development or amenorrhea in females are among the presenting signs during adolescence (27). Most CHH patients have eunuchoid proportions, which are characterized by arm spans that are 5 cm longer than their height. This is related to the delayed closure of the long bone epiphysis in the absence of gonadal hormones. On average, CHH adolescents attain their mid-parental height (12).

CHH can be associated with nonreproductive phenotypes, and presenting symptoms including anosmia/hyposmia (55-100%), hearing loss (5-15%), mirror movement (19-31%), dental agenesis (NA), and renal agenesis (8-15%), eye movement disorders 3-27%), cleft lip/palate (4-7%), scoliosis (13%) and syndactly, polydactyly, and camptodactyly (5%), all of which can be useful diagnostic clues in the differential diagnosis (2,11,12).

Combined forms may be present with other hormone deficiencies (idiopathic or associated with mutations in *PROP-1, HESX1,* and *LHX5*) and/or could be a component of a genetic syndrome (e.g., Prader-Willi syndrome, Noonan syndrome, CHARGE syndrome, Bardet-Biedel syndrome, Waardenburg syndrome, and Hartfield syndrome) (3,15,19).

Congenital Hypogonadotropic Hypogonadism and Spontaneous Remission

It is believed that lifelong hormone therapy is required to maintain sexual function and secondary sexual characteristics in men with IHH (28). Even though the precise pathophysiological mechanisms are unknown, it should be noted that the onset of puberty may occur spontaneously in 10 to 20% of cases later in life, more frequently in males (12). However, there are currently no definitive clinical parameters for predicting the reversibility of CHH. Hence, the cases should be evaluated every two years for the reversibility of the HPG axis (12,29). It should be emphasized that the restoration of reproductive axis function may be temporary, as some people may relapse into GnRH deficiency. Thus, long-term monitoring of reproductive function is necessary (12). Pathogenic variations that induce CHH and reversibility in clinical follow-up can be identified as occurring in *FGFR1* (13%), *GnRHR* (8%), *TACR3* (8%), *PROKR2* (5%), *TAC3* (3%), and *HS6ST1* (3%) (26,29).

Acquired Hypogonadotropic Hypogonadism

The acquired causes of hypogonadotropic hypogonadism may be related to infections (e.g., tuberculosis, meningitis), tumors (e.g., craniopharyngiomas, germinomas), infiltrative diseases (e.g., sarcoidosis, hemochromatosis), autoimmune diseases, radiotherapy, surgery, trauma, drugs, and be functional or idiopathic (Table 1) (30).

Constitutional Delay of Growth and Puberty

CDGP is the most common cause of delayed puberty in both sexes. It accounts for 65-73% of boys and 30-43% of girls (17). It is self-limited and has classically been described as representing late variants of the normal spectrum of pubertal timing (15,19). CDGP also has a substantial genetic component, with 50% to 80% of cases reporting a family history of delayed puberty, frequently in an autosomal dominant fashion (8,9,15,17,19). Although the exact etiological cause of CDGP is not known, increased total energy expenditure and insulin sensitivity are among the possible causes. As linear development slows in comparison to peers entering puberty, the growth chart may indicate a steady downward crossing of centiles. The development of pubic hair (adrenarche) may also be delayed in CDGP, in contrast to CHH, when adrenarche occurs at the normal population age (15,19). Puberty begins at a later age than usual but continues spontaneously (3). The tempo of pubertal development does not follow the chronological age but is concurrent with the bone age, which is delayed compared to chronological age (3,17). It is a self-limited normal variant and is considered an exclusion diagnosis (12,31,32). It is often clinically difficult to distinguish adolescents with CDGP from those with a form of permanent HH. Differentiating between these conditions is particularly difficult in the initial evaluation because adolescents with both conditions are often prepubertal on examination and have low levels of gonadotropins (LH and FSH).

The diagnosis of CHH is the most difficult clinical situation, especially when the clinical presentation overlaps with CDGP and gives no diagnostic signs. The "gold standard" for distinguishing between these two conditions is clinical monitoring until the age of 18 years for signs of endogenous activation of the HPG axis (progressive testicular enlargement or breast development). The presence of endogenous, progressive pubertal development by the age of 18 years is the "gold standard" for differentiating CDGP from IHH (33).

Functional Hypogonadotropic Hypogonadism

FHH can be due to an underlying systemic illness (e.g., celiac disease, asthma, cystic fibrosis), endocrinopathy (e.g., growth hormone deficiency, hypothyroidism,

hyperprolactinemia), medications (e.g., antipsychotics, some antidepressants, opioids), intense exercise, or excessive weight loss (12,20,34). While the underlying etiology can be identified in only 20% of all cases, it is seen more frequently in girls and typically displays reversibility once the underlying pathology is treated or restored (2). The treatment approaches for selective cases are discussed later in this review.

Transient FHH accounts for 10-20% of cases diagnosed with delayed puberty and is likely to affect more girls than boys (Table 1). Nutrition has been suggested to play an important role in the control of GnRH secretion by a mechanism that has not yet been identified. Suboptimal nutritional status results in a hypogonadotropic state and the arrest of pubertal maturation. In malnutrition and chronic diseases, weight loss below the level of 80% of the ideal body weight can cause delayed or arrested pubertal development (9). This pathophysiological condition is usually observed in female patients with anorexia nervosa or excessive physical activity. Chronic disorders, such as sickle cell anemia, thalassemia, cystic fibrosis, inflammatory bowel disease, celiac disease, and chronic renal disease may also be associated with delayed puberty (Table 1). Malnutrition also contributes to short stature, decreased bone mineral density (osteopenia and osteoporosis), and a low mood, which are often observed in these patients (9).

A history of abdominal pain, constipation, or diarrhea, indicating a gastrointestinal disorder; weight gain/loss or temperature intolerance, indicating a thyroid disorder; disordered body image or eating, indicating a restrictive eating disorder; significantly slowed growth, indicating a growth hormone deficiency; or participation in high-demand athletics (gymnastics, ballet, or long-distance running), could be suggestive of an underlying etiology for FHH. On physical examination, children with FHH may be underweight for their height or have physical signs consistent with a specific illness (for example, goiter or abdominal distention) (15,33).

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism, characterized by high levels of gonadotropins, is typically caused by primary gonadal insufficiency. It may develop due to congenital and acquired causes (9,15,33). Conditions associated with primary gonadal failure are listed in Table 1.

Congenital Hypergonadotropic Hypogonadism

Permanent and transient forms of CHH are recognized. CHH may result as a consequence of several etiologies, such as genetic or chromosomal abnormality syndromes (e.g., Turner syndrome in girls or Klinefelter syndrome in boys, Noonan syndrome, Fragile X syndrome, trisomy 13), metabolic disorders (e.g., galactosemia), steroidogenesis defects (e.g., 5-alpha reductase type 2 deficiency, 17-hydroxylase deficiency, aromatase deficiency), FSH and LH β subunit mutations, FSH and LH receptor mutations, androgen defects (e.g., complete androgen insensitivity syndrome), vanishing testes syndrome (in boys), and autoimmune oophoritis (in girls) (Table 1) (3,9,35).

Klinefelter syndrome (47, XXY or 48, XXXY) seen with a prevalence of 1 in 660 males, is the most common chromosomal aneuploidy and primary hypogonadism etiology in boys. Most affected people naturally reach puberty at a normal age, but in the years that follow, testosterone levels progressively drop as pubertal arrest occurs and seminiferous tubule degeneration is accompanied by Leydig cell degeneration in Tanner stages 4-5. Individuals with Klinefelter syndrome usually present with tall stature, gynecomastia, behavioral and neurocognitive problems, and their testicular volumes are usually less than 5-6 mL.

Turner syndrome is the most frequent type of hypergonadotropic hypogonadism in females, occurring in 1 in 2,000 to 2,500 live births. Individuals present with specific phenotypical and clinical characteristics (e.g., facial appearance, neck webbing, short stature, cardiovascular, skeletal, and renal anomalies) and are diagnosed in the presence of one intact X chromosome with complete or partial absence of the other chromosome. The 45, X karyotype is seen in almost half of all girls with Turner syndrome. Puberty is frequently missing or delayed in Turner syndrome, and it is followed by progressive ovarian failure. Importantly, up to 30% of females will experience spontaneous pubertal growth, and 2% to 5% will experience spontaneous menstruation (9).

Acquired Hypergonadotropic Hypogonadism

Acquired defects may be due to radiation, chemotherapy, autoimmunity (e.g., autoimmune polyglandular syndrome), gonadal infections (e.g., mumps), or idiopathic (Table 1).

Diagnostic Evaluation of a Patıent with Delayed Puberty

The diagnostic evaluation should consider the medical history, including height and weight charts, developmental milestones, nutritional status, medications (chemotherapy, radiation, steroid), history and/or symptoms of chronic disease, and psychosocial functioning, trauma, or infection, family history, and physical examination (current height, weight, pubertal staging, and syndromic features) (15,36). Symptoms suggestive of thyroid dysfunction, androgen excess, hyperinsulinism, or an underlying chronic disease should be evaluated carefully to exclude the diagnosis

Table 1. Etiology of delayed puberty

	Hypergonadotropic hypogonadism		Permanent hypogonadotropic hypogonadism		Transient hypogonadotropic hypogonadism	
					Functional hypogonadotropic hypogonadism	CDGP
Frequency						
Girls	**15-25%**		**10-20%**		**20-30%**	**30-55%**
Boys	**5%**		**10%**		**10-20%**	**60-80%**
	Congenital	**Acquired**	**Congenital (>30 gene implicated)**	**Acquired**	• Systemic illness or infections ✓ AIDS	
	• Genetic syndromes and related disorders ✓ Noonan syndrome ✓ Klinefelter syndrome ✓ Down syndrome ✓ Fragile X syndrome (FMR1)	• Trauma • Testicular torsion	• Isole or multiple PHD ✓ Anosmic (Kallmann syndrome) ✓ Normosmic (Isolated) ✓ HPG axis developmental disorders (Rathke's pouch cyst) • Monogenic obesity (LEP, LEPR, and PCSK1) • Syndromic obesity ✓ Prader-Willi syndrome ✓ Bardet-Biedl syndrome CHARGE syndrome • Midline defects ✓ Sept-optic dysplasia ✓ Congenital hypopituitarism	• CNS ✓ Tumors/infiltrative diseases ✓ Astrocytoma ✓ Germinoma ✓ Glioma ✓ Craniopharyngioma ✓ Prolactinoma ✓ Langerhans cell histiocytosis ✓ Sarcoidosis	• Rheumatic disease ✓ Juvenile rheumatoid arthritis • Respiratuvar disease ✓ Asthma • Renal disease ✓ Chronic renal disease • Hematologic and oncologic disease ✓ Sickle cell disease ✓ Hemosiderosis ✓ Thalassemia ✓ Langerhans cell histiocytosis ✓ Leukemia and lymphoma	
	✓ Gonadal dysgenesis ✓ Turner syndrome (45, X, or mosaic) ✓ 46, XX pure gonadal dysgenesis	• Chemotherapy		• Prior CNS infection Meningitis Encephalitis	• Endocrinopathy ✓ Diabetes mellitus ✓ Hypothyroidism ✓ Hyperandrogenism ✓ Hyperprolactinemia ✓ Growth hormone deficiency ✓ Hypercotisolism	
	• Testicular regression syndrome (Anorchia)	• Radiation therapy		• Radiation therapy	• Gastrointestinal disease ✓ Cystic fibrosis ✓ Celiac disease ✓ Inflammatory bowel disease ✓ Hepatic disease	
	• Defects in steroidogenesis ✓ 5-alpha reductase deficiency ✓ 17, 20 lyase deficiency Congenital lipoid adrenal hyperplasia (StAR) ✓ 17-hydroxysteroid dehydrogenase deficiency • Resistance to androgen receptor • Sertoli cell only syndrome • Gonadotropin resistance	• Gonadal infection ✓ Mumps, ✓ Coxsackie		• Chemotherapy	• Excessive exercise	
	• Metabolic disease ✓ Galactosemia	• Autoimmune orchitis • Autoimmune oophoritis		• Trauma	• Malnutrition • Anorexia nervosa/bulimia	
		• Gonadectomy		• Cranial surgery	• Drug (eg.glucocorticoid)	

CNS: central nervous system, CDGP: constitutional delay of growth and puberty, AIDS: acquired immune deficiency syndrome, HPG: hypothalamic-pituitary gonadal

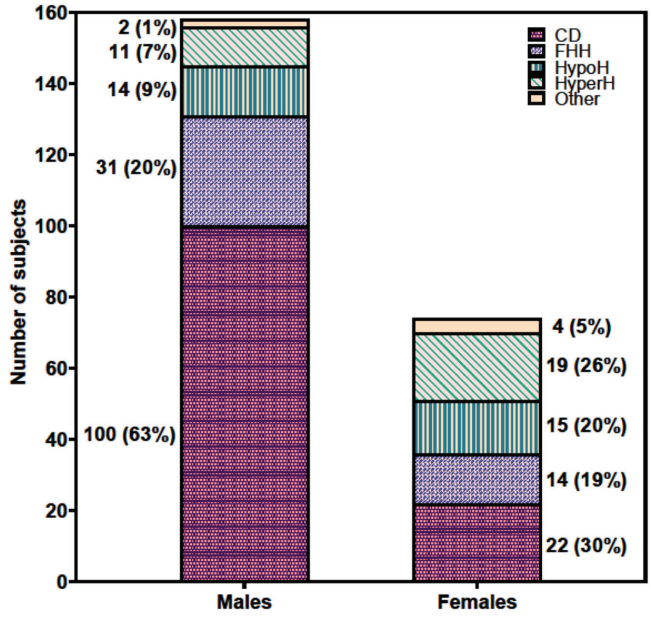

Figure 1. Distribution of diagnostic categories among males and females (20)

CD: constitutional delay of growth and puberty, FHH: familial hypogonadotropic hypogonadism, HypoH: hypogonadotropic hypogonadism, HyperH: hypergonadotropic hypogonadism, Other: etiology not clearly classified

of FHH. While hypogonadism is typically diagnosed during adolescence, patients with features suggestive of hypogonadism, such as micropenis and bilateral undescended testis in boys, or those with hypogonadism in the family history, can also be evaluated during mini-puberty (2). A thorough history should include evidence of anorexia and the intensity of exercise. A comprehensive family history is essential, including childhood growth trends, age at pubertal onset of both parents and siblings, and any history of infertility, anosmia, and midline abnormalities of parents and siblings (9).

Physical signs, such as cleft lip or palate, bimanual synkinesia, congenital ptosis and abnormal visual spatial attention, abnormal eye movements, sensorineural hearing loss, unilateral renal genesis, agenesis of one or more teeth (hypodontia), obesity, features suggestive of CHARGE syndrome, and digital and other skeletal abnormalities, will raise suspicion of the diagnosis of CHH. A genetic condition may be involved if there is delayed cognitive development accompanied by obesity or dysmorphic traits (9).

A bone age assessment, early morning basal testosterone, LH, and FSH levels (to detect hypergonadotropic hypogonadism), and a biochemical analysis that includes a full blood count should all be part of the initial screening process for delayed puberty. It is advised to do tests for sedimentation rate (or C-reactive protein), renal and liver function, thyroid function, electrolytes, celiac screen (anti-transglutaminase IgA), insulin-like growth factor-1 (IGF-1), and prolactin to rule out any additional pituitary hormone insufficiency or underlying chronic illness (15). Serum testosterone levels show a diurnal rhythm, with a decrease in the afternoon and evening. As a result, blood samples should be taken at the same time every day (ideally in the morning) (37).

Other tests, such as pelvic ultrasound for gonad and uterine evaluation and renal ultrasound in X-linked CHH, may be necessary due to probable anosmin (*ANOS*) mutations associated with renal malformation or unilateral agenesis (9).

Olfactory function is a hallmark of the clinical assessment of CHH, as ~50% of patients have a defect in the sense of smell or KS, also known as "olfactogenital dysplasia" (12). Both objective (Pennsylvania Odor Test) and subjective (detailed interview) olfactory tests should be performed (9). Self-reporting anosmia is sensitive and specific, whereas self-reporting normal olfaction is unreliable. Therefore, formal olfactory testing should be performed in all patients with CHH (12).

Magnetic resonance imaging (MRI) plays a significant role in diagnosing hypogonadism. Brain MRI can be used to rule out an acquired form of hypogonadism, such as a central nervous system tumor, and to identify features of CHH, such as defects in the olfactory bulbs, corpus callosum, semicircular canals, and cerebellum. In association with anosmia or hyposmia, patients with KS typically present with unilateral or bilateral olfactory bulb agenesis, olfactory tract agenesis, and/or gyrus malformation. An MRI should be obtained for any patient with suggestive clinical features of intracranial pathology (12,15).

In the presence of hypergonadotropic hypogonadism, a karyotype analysis, or comparative genomic hybridization (to identify lesser levels of mosaicism) can be used to diagnose Turner or Klinefelter syndrome (15).

A spermiogram is the quantitative and qualitative analysis of semen for the assessment of an adult man's fertility potential (12). However, it is not recommended in routine practice to perform spermiograms at certain intervals (every 3-6 months) during the physiological puberty induction protocol in adolescents. In the second or third year of physiological induction therapy, a spermiogram may be carried out for those who are curious about the fertility status or for cryopreservation (banking) of sperm (38). According to a meta-analysis, gonadotropin therapy resulted in a mean sperm concentration of 5.2 million/mL [95% confidence

interval (CI), 4.7-7.1]. The median time to achieve sperm in the ejaculate was 7.1 months (95% CI, 6.3-10.1), and the median time to conception was 28.2 months (21.6-38.5) (39). The latest World Health Organization criteria for semen analysis interpretation were published in 2010. They were based on semen samples from >4500 men in 14 countries and defined lower reference limits for the following parameters: 1.5 mL for semen volume, 15 million/mL for sperm count, 40% for total motility, and 4% for normal morphology (40).

Elevated basal plasma FSH and LH levels in the early morning (FSH level >25 IU/L to >40 IU/L) indicate hypergonadotropic hypogonadism, while undetectable, low, at the lower limit of normal levels should suggest hypogonadotropic hypogonadism (3,41,42). The differential diagnosis of CDGP and CHH does not benefit from baseline gonadotropin values, but basal gonadotropin levels are frequently elevated in primary hypogonadism because of conditions like Turner or Klinefelter syndrome (9).

Over the past 30 years, various basal and stimulation tests have been proposed to differentiate between adolescents with CDGP, FHH, and persistent HH. Basal gonadotropins, GnRH, and human chorionic gonadotropin (hCG) stimulation tests all have limitations in diagnostic specificity and sensitivity to differentiate between the groups (2,42). Basal FSH and LH levels can be identified at prepubertal levels (3). In addition, low total testosterone levels in boys (free testosterone should be calculated if sex hormone-binding globulin is below the reference range) or low estradiol levels in girls may suggest the presence of hypogonadism (1,43). Since sleeping patterns, food consumption, acute illness, and immunoassay type may profoundly affect the values measured, a single cut-off for assessment cannot be given; however, levels of total testosterone >20 ng/dL in boys and >12 pg/mL in girls indicate the pubertal onset, while >12 nmol/L (>346 ng/dL) for testosterone and >50 pg/mL for estradiol have been suggested to rule out the diagnosis of hypogonadism (3,44). An 8 a.m. testosterone level >20 ng/dL predicts the onset of puberty within 12 to 15 months. At testosterone levels >100 ng/dL, structural growth is accelerated (42).

Levels of FSH, LH and total testosterone stimulated by dynamic testing using GnRH, GnRH analog (buserelin, leuprolide, nafarelin, triptorelin), and hCG can be useful (3). However, the diagnostic utility of these tests in distinguishing between youth with CHH and CDGP is limited due to significant overlap in diagnostic thresholds (15). However, in the absence of a standardized protocol, threshold values (peak LH, FSH, and total testosterone) vary widely and reliability is low (33). A predominant LH response in comparison to FSH or peak LH levels >5 IU/L in the GnRH test can indicate pubertal onset or FHH/CDGP (45). However, prepubertal GnRH test response should not rule out the diagnosis of CDGP and FHH (33). Furthermore, it should be noted that while a stimulated peak LH level above 5 IU/L (as measured by immunochemiluminometric assays) may suggest a diagnosis of CDGP, a normal response may still be observed in cases of partial HH.

Minipuberty provides a window of opportunity for evaluation of the functionality of the HPG axis before puberty for infants with CHH (7). As serum placental estrogen levels decline during the first postnatal week, increasing pulsatile GnRH production leads to increased gonadotropin and sex steroid levels in both sexes (12,42). Gonadotropin levels in healthy infants start to increase during the first week of life and then decrease toward the age of six months, except for FSH levels in girls that remain elevated until 3-4 years of age (9). In the neonatal period, low (1.2 IU/L) or undetectable levels of FSH are suspicious findings and may indicate CHH (2,46). During childhood, the diagnosis is difficult due to the physiological hypogonadism normally present during this period. The gonadotropic axis is resting, and LH is only detectable by ultrasensitive assays, whereas FSH plasma concentrations are variable (9). In adolescents, total plasma testosterone is low for the age, and baseline FSH and LH are also low or low normal. The response to the GnRH test is variable and depends on the severity of the gonadotropin deficiency, e.g. it may show no response in profound HH but be normal in partial HH (42).

Incorporating the markers of gonadal function, such as Inhibin B concentrations (marker of Sertoli cell), INSL3 concentrations (marker of Leydig cells), and anti-Müllerian hormone (AMH) levels (marker of granulosa cells and Sertoli cell) can assist in confirming the diagnosis (2,47,48). While the relationship between testosterone level and AMH is positively correlated due to low AR expression (2-15%) in Sertoli cells in the first four years of life, this relationship reverses with the increase in AR expression in the pubertal period. After the age of eight years, AR expression in Sertoli cells reaches 90% and spermatogenesis can be induced by the effect of increasing intratesticular testosterone concentration in the pubertal period (8). In the pubertal period, total testosterone level is positively correlated with inhibin B and negatively correlated with AMH levels. Normogram values of AMH and inhibin levels were determined according to age and sex (8,12,42). Low AMH levels might be indicative of ovarian failure (49).

Recent studies suggest that inhibin B may be an informative and simple first-line test. Inhibin B levels, controlled through FSH, reflect Sertoli cell number and activity. Serum inhibin

B levels correlate well with testicular size, and low inhibin B levels are a negative predictor of fertility (12). Despite considerable diversity in the threshold level of inhibin B to differentiate between CHH and CDGP, preliminary investigations evaluating the significance of baseline inhibin B concentrations were encouraging but have not been verified. Undetectable inhibin B (< 10 pg/mL) is considered diagnostic of anorchia, but low, close to undetectable levels are also seen in severe forms of CHH. Its levels correlate with testicular volume and are therefore a good marker of the spermatogenesis and severity of HH. Inhibin B transiently peaks around 2-4 months after birth, decreases during childhood, and increases again during puberty (50). Rohayem et al. (50) observed that a threshold value of ≥28.5 pg/mL for inhibin B was sufficient to distinguish CDGP from HH in male patients, with a sensitivity of 95%, and specificity of 75%. Coutant et al. (48) showed in genital stage 1 that a single inhibin B level of 35 pg/mL or less had a sensitivity and specificity of 100% and a positive predictive value (PPV) of 93% for distinguishing persistent HH patients from CDGP patients. In another study, the PPV was 73% when the inhibin B threshold was set at 100 pg/mL. The predictive value increased to 100% when only patients with persistent HH with a testicular volume of less than 3 mL were considered. However, the sensitivity and specificity of inhibin B were reported to be lower when comparing patients with HH diagnosed as part of multiple pituitary hormone deficiency with the CDGP group. Combined markers have also been used to differentiate CDGP and persistent isolated HH. Combined markers have also been used to differentiate CDGP and persistent isolated HH. Binder et al. (51) reported that a combination of basal LH and inhibin B provided 100% sensitivity and 98% specificity for discrimination of the two conditions when basal LH < 0.3 U/L and inhibin B < 111 pg/mL were used as combined decision limits. Although the exact role of inhibin B during female puberty is not known, its increasing serum concentration at early puberty reliably reflects the secretory maturation of the ovarian follicles, which is driven by gonadotropins.

Inhibin B is secreted by granulosa cells in women and is a marker of the number of antral follicles. Very few studies have investigated the levels of circulating inhibin B levels in females with CHH (12). There have been a limited number of studies in female patients, and the threshold value for inhibin B in terms of discriminating CDGP and HH was determined to be < 20 pg/mL in the study by Binder et al. (51).

In pubertal patients with central hypogonadism, AMH is low for the Tanner stage - reflecting lack of FSH stimulation - but high for age - reflecting lack of testosterone inhibition (52). When compared to prepubertal levels, the AMH decrease during Tanner stages 2 and 3 coincides with the increase in intratesticular testosterone and the meiotic onset of germ cells in the seminiferous tubules during puberty. Although AMH serum levels have been reported to be a useful marker in discriminating CDGP and HH in male patients, they are not as discriminative as inhibin B (8,50). Serum prolactin, free T4, thyroid-stimulating hormone, cortisol, IGF-1, and IGF binding protein-3 may be determined to characterize combined pituitary hormone deficiencies (2).

Treatment of Hypogonadism

Hormonal therapy is used to induce puberty in adolescent males based on published consensus and expert opinion. However, there are currently no evidence-based guidelines regarding the optimal timing and regimen for inducing puberty in either males or females (53,54,55,56).

The choice of preparation and administration route for estrogen or testosterone is based on the advantages and disadvantages of the available regimens (12). Among the different forms of testosterones, oral forms have the disadvantage of shorter half-lives, transdermal forms may cause skin reactions, and subcutaneous implants require a surgical intervention (57,58).

Treatment Approach in Transient Hypogonadism

Delayed puberty can cause psychological distress and low self-esteem in adolescent males. It also negatively affects metabolic profile, fat distribution, muscle mass, bone mass, and growth. It is important to address this issue promptly to prevent further complications (53). Therefore, for individuals with a possible diagnosis of FHH or CDPG, it is recommended that puberty be induced in the short term by low-dose administration of sex steroids. In addition, induction of delayed puberty can help to trigger a pubertal "jump-start" or confirm the diagnosis of a permanent or transient etiology (59).

Management of Constitutional Delay of Growth and Puberty

Although the "watchful waiting" strategy is one of the main approaches in CDGP, puberty can be induced with low doses of testosterone and estrogens when chronological age reaches 14 years and bone age reaches 12 years in boys and chronological age reaches 13 years and bone age reaches 11 years in girls (3,32,60). Inductions are administrated for a cycle of 3-6 months, followed by a 3-6-month window period of clinical follow-up to allow pubertal "jump start" (60). If progression fails, a second trial with higher dosages can be administered, before commencing lifelong hormone replacement treatment (14). Parenteral testosterone is

commonly used to induce puberty in boys with hypogonadism and CDGP due to its flexible dosing administration (53). In girls, 17-β-estradiol (oral or transdermal), ethinylestradiol (oral), or conjugate equine estrogens (oral) are available to induce puberty (32,60). The dosing equivalents of various estrogen preparation vary significantly: 0.1 mg transdermal 17-β-estradiol equates to 2 mg oral 17-β estradiol, 20 mg oral ethinyl estradiol or 1.25 mg oral conjugated estrogen (61). The typical starting dose of estrogen is 0.25 to 0.5 mg oral 17-β-estradiol (or 5 micrograms/kg) daily. Alternatively, if the transdermal route is preferred, 3.1 to 6.2 mg (1/8 to 1/4 of a 25 mg/24 h 17-β-estradiol patch) can be used (15). The recommendation for pubertal induction protocols in cases with suspected CDGP is summarized in Figure 2 for male subjects and Table 2 for female subjects (60).

The use of aromatase inhibitors (anastrozole or letrozole) for a six-month duration has been shown to induce puberty and accelerate growth in boys (32,62). Mauras et al. (63) demonstrated that oral letrozole (2.5 mg/day) or anastrozole (1.0 mg/day) may be a viable alternative treatment option to intramuscular testosterone therapy for pubertal induction in male patients with CDGP after six months of use (11,62).

Management of Functional Hypogonadotropic Hypogonadism

Although there is no clear consensus on the general approach to FHH, the underlying etiological cause should be treated primarily (17). In these patients, the process normalizes spontaneously when the energy deficit is corrected or the underlying disease is treated (17). Delayed puberty is also frequently observed in chronic renal failure. However, spontaneous recovery of gonadotropin secretion has been observed in these patients after successful renal transplantation (9). In FHH, if attempts to modify nutritional, psychological, and exercise-related variables are unsuccessful in establishing menses, clinicians may consider estrogen replacement. Even after as little as 6-12 months of amenorrhea, bone outcomes may be compromised. Therefore, clinicians may consider short-term transdermal E2 with cyclic oral progestin therapy after 6 to 12 months of nutritional, psychological, and exercise-related interventions in those with low bone density and/or evidence of skeletal fragility. It should be noted that E2 replacement therapy may not protect bone health if there are ongoing nutritional factors or energy deficits (36).

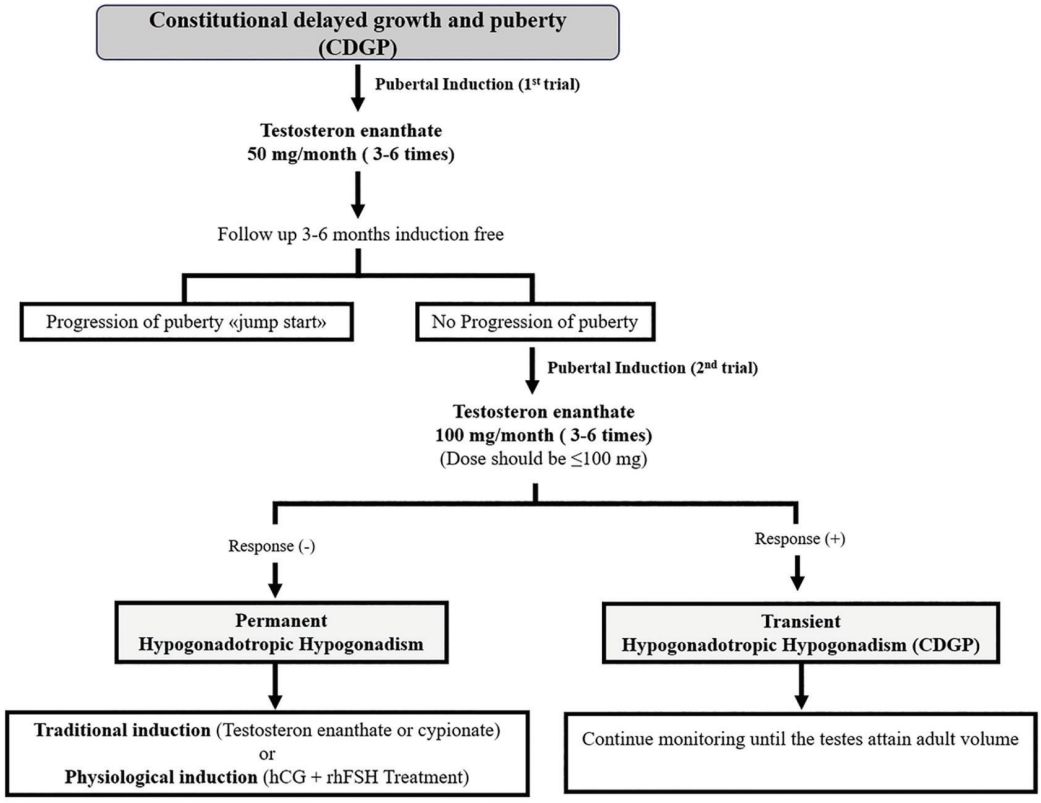

Figure 2. Pubertal induction protocol in boys with CDGP

CDGP: constitutional delay of growth and puberty, hCG: human chorionic gonadotropin hormone, rhFSH: recombinant human follicular stimulating hormone

Table 2. Pubertal induction protocol in girls with CDGP

	Route	Dose	Duration	Start
Ethinyl estradiol	PO	0.05-0.1 µg/kg/day (2.5 µg/day for 6-12 months. Increase after 6 months to 5 ug/day if necessary	Until breast development reaches B3	≥11-12 years BA ≥13 years CA
Conjugated estrogens	PO	0.3 mg on alternate days for 6-12 months. Increase after 6 months to 0.3 mg/day if necessary		≥11-12 years BA ≥13 years CA
17-β-estradiol	PO	5 ug/kg/day Increase after 6 months to 10 ug/day if necessary		≥11-12 years BA ≥13 years CA
	T.D	As shown in Figure 3		≥11-12 years BA ≥13 years CA

IM: Intramuscular, SC: subcutaneous, BA: bone age, CA: chronological age, PO: per oral, TD: trans-dermal, CDGP: constitutional delay of growth and puberty

Treatment of Permanent Hypogonadism

Approach to Hypogonadism in Male Patients

There are two possible approaches to pubertal induction in boys. The first is parenteral or transdermal testosterone esters, which are used in the treatment of both hypergonadotropic and hypogonadotropic hypogonadism (traditional pubertal induction). The second is GnRH and gonadotropin therapy (physiological pubertal induction), which is recommended in the treatment of permanent HH (38).

Parenteral Testosterone Replacement Treatment

Adolescents with delayed puberty should start puberty induction therapy around the average age of normal male puberty (12 years). In cases where the distinction between permanent HH and CDGP can not be made, it is recommended to wait until the chronological age is 14 and the bone age is 12 before starting testosterone replacement treatment (TRT) (53,60,64). In addition, in prepubertal children who are short for their age, postponing treatment may be an option to increase their final height (53,65). The most commonly used method of exogenous TRT is known to induce virilization, enhance sexual function, increase bone density, and promote lean body mass. Gonadal development cannot be stimulated via TRT, since it suppresses serum LH secretion, decreasing intratesticular testosterone levels by 98%. Some authors argue against its use as a therapeutic option for hypogonadal males, and favor gonadotropins, since it may cause atrophy of the germinal epithelium and decrease spermatogenesis (7,66,67,68). Thus, in adolescent males with permanent HH, hCG, with or without FSH, appears to be more physiological and potentially safer than testosterone in initiating spermatogenesis and testicular growth (53). A meta-analysis by Rastrelli et al. (69) found no significant difference in sperm count in patients receiving TRT before gonadotropin treatment (5.84 million/mL vs. 4.88 million/mL, $p = 0.684$). This lack of association implies that previous testosterone exposure may not exert an adverse effect on fertility rates. However, the authors also underlined the impact of the potential interferences in the interpretation of data, such as the ecological fallacy, or the availability of small numbers of cases. Furthermore, the potential reversibility of the cases included in the analysis was also highlighted, since 20% of the >300 patients with hypogonadism have shown reversibility (70).

In adolescent males with CDGP or permanent hypogonadism, TRT is the most commonly used therapy to induce puberty. Compared with other treatments, testosterone is an effective, convenient, safe, well-tolerated, and cost-effective option (53). Currently, the only formulations approved by the US Food and Drug Administration for delayed puberty are intramuscular testosterone esters, particularly testosterone enanthate, cypionate, undeconate, and subcutaneous testosterone pellets (71,72). In addition, several new formulations, including transdermal, nasal, subcutaneous, and oral formulations, have recently been developed to improve the pharmacokinetic profile and ease the administration route, thereby increasing patient compliance in adult males with hypogonadism (Table 3) (53,71). However, during the early pubertal period, parenteral testosterone is preferred due to the difficulty of dose titration with other forms of testosterone. All these formulations are not approved for the pediatric age group, although some of them are used as "off-label" regimens (71).

The most commonly used form of TRT is intramuscular injection of testosterone esters. Unmodified testosterone has a half-life of only 10 minutes and would have to be injected very frequently. Esterification of the testosterone molecule at position 17, for example with propionic or enanthic acid, prolongs the activity of testosterone in proportion to the length of the side chain when administered intramuscularly (37,73). Intramuscular injections of these testosterone esters (testosterone propionate and testosterone enanthate) result in supraphysiological testosterone levels early after administration and subphysiological levels near the end of the dosing interval. Attempts have been made to overcome

Table 3. Available preparations and dosing strategies for patients with permanent hypogonadism

Preparation and route of administration			Initial dosage (pubertal induction dose)	Dose increment, interval	Adult dose
Induction of puberty in boys					
Testosterone enanthate, cypionate, or a mixture of testosterone esters (IM)			25-50 mg/ every 4 weeks or 1 mg/kg/ every 4 weeks	Increase of 50 mg every 3-12 months (ideally 6 months) until the dose of 150-200 mg every 4 weeks	150-200 mg/every 2 weeks
Testosterone undecanoate (IM)			No data available	No data available	750-1000 mg/every 10-14 week
Testosterone undecanoate (oral)	20-40 mg/day	Every 6 months	40-80 mg/day 2 twice daily		
Testosterone gels, 1 % or 2 % (transdermal)	1 % gel 0.5 g up to 5 g daily 2 % gel 10 mg daily for 3 months	No data available	1 % gel: 50-100 mg daily 2 % gel: 40-70 mg daily		
Testosterone patch (transdermal)	Age 12.5-15 years 2.5-5.0 mg for over 8-12 h/daily for 8 weeks	5 mg for 8-12 h/ daily application for 6 months	Adult dose: 5-10 mg over 24 h daily		
Subcutaneous testosterone pellets			No data available	No data available	Adult dose: 8-10 mg/kg every 6 months three doses (or 150-450 mg every 3-6 months
Induction of puberty in girls					
Ethinyl estradiol, oral	0.05-0.1 µg/kg/day (2.5 µg/day)		Every 6-12 months		10-20 µg/day
17-β-estradiol, oral	5 µg/kg/day (0.25 mg/day)		5 µg/kg, every 6 -12 months		1-2 mg/day (max 4 mg)
17-β-estradiol, transdermal*	0.08-0.12 µg/kg/day for 10 hours		Detailed in Figure 5		50-100 µg/day twice a week

*The transdermal treatment protocol is detailed in Figure 5.
IM: intramuscular, max: maximum

this effect by combining short- and long-acting esters (e.g. Sustanon, Testoviron Depot). However, it has been observed that these products result in even higher initial serum levels of testosterone, without any corresponding increase in the duration of their effects. Testosterone propionate must be administered every 2-3 days, whereas testosterone enanthate and testosterone cypionate only need to be administered every 2-3 weeks (72). Although long-acting testosterone undeconate has been reported as safe for continuing pubertal induction after the age of 18 years, there is no data on its use in children (74,75).

The lower limit of 'normal' serum testosterone concentration is controversial, and the generally used or suggested lower serum testosterone concentration for starting therapy varies in the four European countries (Germany, France, the UK, and Spain) surveyed by the authors. These lower thresholds range from 216-346 ng/dL. According to research, serum total testosterone concentration of 300 ng/dL (10.4 nmol/L) may be clinically relevant for starting TRT in patients with symptoms of permanent hypogonadism (37).

During puberty, TRT should be increased gradually to mimic normal pubertal physiology and can be stopped when the HPG axis is significantly activated, as indicated by an increase in the testicular volume of 6 to 8 mL (53,71). In adolescents with permanent hyper- or hypogonadism, it is recommended to initiate treatment with a low dose of intramuscular testosterone enanthate or cypionate (25-50 mg every four weeks or 1 mg/kg per month) and gradually increase the dose by 50 mg every 6-12 months over a period of 2-3 years. After reaching a monthly dose of 150-200 mg, the dosing interval is increased to every 2 weeks. The recommended adult dose is 150-200 mg every two weeks. Table 3 summarizes the doses of pubertal induction with different testosterone products (11,12,59,62,71,76). The Endocrine Society Clinical Practice Guidelines recommend testosterone enanthate or cypionate 75-100 mg/week or 150-200 mg/two weeks for young adults and adult with hypogonadism (73,77). To prevent accelerated bone age and short final adult height, it is recommended to avoid high-dose testosterone therapy at the start of pubertal induction (76). Continuous monitoring of endogenous

puberty is recommended. Testicular volume can be assessed every six months, and testosterone and LH levels can be measured one month following the most recent injection. If endogenous puberty does not occur by the age of 18 years, the diagnosis of permanent HH is established (15).

Sustanon (250 mg of Sustanon corresponds to 176 mg actual testosterone) is the most commonly used, commercially available form of testosterone ester mixture, consisting of testosterone propionate (30 mg), testosterone phenylpropionate (60 mg), testosterone isocaproate (60 mg), and testosterone decanoate (100 mg). Various testosterone esters exhibit distinct elimination half-lives throughout the body (44,64). A single dose of Sustanon 250 mg leads to an increase of total plasma testosterone with peak levels of approximately 70 nmol/L (2019 ng/dL) (C_{max}), which is reached approximately 24-48 h (t_{max}) after administration. Plasma testosterone levels return to the lower limit of the normal range in males in approximately 21 days (https://www.medicines.org.uk/emc/product/5373/smpc#gref) (78). The actual testosterone content in 100 mg of testosterone enanthate and cypionate is 70 and 73 mg respectively, while 100 mg of Sustanon contains a similar amount (70.4) of actual testosterone. Table 4 summarizes the peak effects, half-lives, and actual testosterone amounts within 100 mg of parenteral testosterone products (73,74,75,79,80,81).

Infancy

To date, hormone therapy during the neonatal period has only been used in male patients with micropenis/cryptorchidism and HH in the neonatal period (12). In infancy, cryptorchidism in males should be corrected by orchiopexy at 6-12 months of age to preserve future fertility potential (2,82). There are currently no additional data on the use of hCG or GnRH supplements during the minipubertal period for future fertility. Some publications suggest that high-dose hCG may have negative effects on germ cells, including increased apoptosis, intratesticular hemorrhage, inflammation, and potential harm to future fertility (12,83,84). On the other hand, it has been reported that hCG and GnRH treatments have a beneficial effect on increasing penis size, increasing testicular volume, and facilitating descent of undescended testicles in the minipubertal period, although the negative effect on the testes remains controversial (12,85,86,87). Smaller doses of FSH (2.5 IU/kg twice a week) and hCG (20 IU/kg twice a week) have been recommended during infancy; however, larger prospective randomized controlled trials are needed (85,88). In addition to this treatment, parenteral TRT can increase the size of the penis in boys with central hypogonadism and primary hypogonadism-associated micropenis (2,76,89). Administration of testosterone cypionate or enanthate in oil (25 mg) every 3-4 weeks for 3-4 months or topical 5α-dihydrotestosterone gel (5%) are two possible approaches (89,90). The 5% testosterone gel can either be applied 3 times a day for 5 weeks or 0.2-0.3 mg/kg once daily for 3 months (76,90,91).

Table 4. Actual testosterone content of testosterone-containing products

Testosterone product	Peak after injection (hours)	Terminal ($t^{1/2}$) Median-residence time (days)	Actual testosterone (per 100 mg)	Actual testosterone content of commercially available products (implementation periods)
Testosterone undecanoate (IM) 750 mg 1000 mg	 240 168	20.9-34.9	61	*Marketed under the brand names Nebido (1000 mg/4 mL) vial (Bayer), IM (10-14-week interval) Total actual testosterone 610 mg
Testosterone decanoate	24-48	12-14	62	*Marketed under the brand names Sustanon (250 mg/1 mL vial) (Organon), IM (2-4 weeks interval) Total actual testosterone 176 mg
Testosterone Isocaproate		7-9	72	
Testosterone phenylpropionate		3-4	66	
Testosterone propionate		0.8-1.5	83	
Testosterone cypionate 200 mg	 48-120	6-7	70	1-4 weeks interval, IM or SC
Testosterone enanthate 100 mg/week 200 mg/2 week 300 mg/3 week 400 mg/4 week	 96-120 48 36-48 36-48	4.5-8.5	 73	1-4 weeks interval, IM or SC

*The Sustanon ampoule (a mixture of testosterone esters) is a parenteral product. The contents of the ampoule are indicated by the grey-shaded boxes.
IM: intramuscular, SC: subcutaneous

Monitoring of Parenteral Testosterone Replacement Treatment

It is recommended to monitor testosterone levels 3 to 6 months after starting TRT. Although there are different recommendations regarding the measurement of testosterone levels (before injection, one week after injection, etc.) due to the different half-lives of testosterone products, the general recommendation is the midpoint of the two injection times (89). For patients receiving long-acting parenteral testosterone therapy, such as testosterone cypionate and enanthate, which have a short half-life of seven days, it is recommended that testosterone levels be measured four weeks after the start of treatment and one week after injection. For testosterone undecanoate, the levels should be measured before each subsequent injection (77).

When the adult dose is attained in the second or third year of treatment, the total testosterone level should be kept within the mid-normal reference range (350-700 ng/dL) (77,92). If testosterone is >700 ng/dL or <350 ng/dL, the dose or frequency should be adjusted. The major disadvantage of parenteral testosterone treatment is wide fluctuation of plasma testosterone levels, which are not in the physiological range for at least 50% of the time. After a single intramuscular injection, serum testosterone levels rise above physiological ranges, then decline gradually into the hypogonadal range by the end of the dosing interval (77). Preparations are generally well tolerated, but they may cause side effects, such as local reactions, gynecomastia, priapism, increased hematocrit (polycythemia), deranged liver function, and inappropriate behavioral changes (38,76). Polycythemia, which is defined as a hematocrit level greater than 52%, is a known side effect of TRT. It is recommended to determine hematocrit levels at baseline, at 3 to 6 months, and then annually. If the hematocrit level exceeds 54%, therapy should be discontinued until the hematocrit level decreases to a safe level (77). Patients should be monitored for the development of this condition and therapeutic phlebotomy may be required if it becomes severe (79). Testosterone esters should be used with caution in cases of renal impairment and avoided in cases of hepatic impairment or hypercalcemia (76).

Physiological Pubertal Induction in the Management of Hypogonadotropic Hypogonadism

TRT aims to induce virilization but does not stimulate spermatogenesis. On the other hand, pulsatile GnRH and gonadotropin treatments for 6-24 months result in testicular growth in almost all individuals and stimulate spermatogenesis in 80-95% of patients without undescended testes (38,87,92). Thus, mimicking the HPG axis during the mini-pubertal period to treat infants with congenital etiologies or to induce puberty at appropriate pubertal ages can be used as an alternative to parenteral TRT (92).

Various physiological pubertal induction protocols, including the use of hCG alone or in combination with recombinant FSH (rFSH), have been proposed in guidelines and studies for adolescent boys with permanent HH (Figure 3) (38,50,53,66,93). Various hCG products, which are derived from pregnant women's urine (Pregnyle, N.V. Organon, The Netherlands, or Choriomon, Institut Biochimique SA, Switzerland) or from recombinant DNA technology (Ovitrelle, Merck Serono) are commercially available, and no difference in efficacy has been reported between the two forms (94,95). One study showed that after administration of urinary hCG (5000 IU) and recombinant hCG (6500 IU), there was no significant difference in peak testosterone and estradiol levels (96). Urinary hCG preparations are currently marketed in lyophilized vials containing 1500 or 10,000 IU for intramuscular use. In contrast, recombinant hCG is available in prefilled syringes or pen devices containing 250 mg of pure hCG equivalent to approximately 6500 IU of urinary hCG. Although Ovitrellin single injection pens (0.5 mL, 250 µg = 6500 IU hCG) are not suitable for physiological pubertal induction, there are ready-to-use pens with adjustable doses that are more practical to use. However, these pens are not widely available in many countries (39). Recombinant hCG is purer than hCG derived from urine and

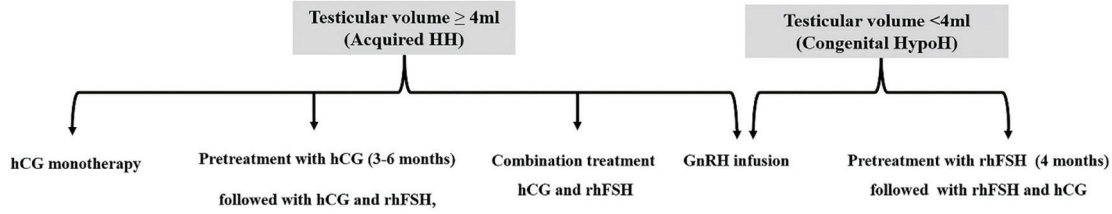

Figure 3. Physiological pubertal induction protocols in hypogonadotropic hypogonadism with gonadotropins

hCG: human chorionic gonadotropin hormone, rhFSH: recombinant human follicular stimulating hormone, HypoH: hypogonadotropic hypogonadism, GnRH: gonadotropin-releasing hormone, HH: hypogonadotropic hypogonadism

has a better quality and safety profile than its counterparts derived from urine (94,95). Patients using urinary hCG for induction of HH may develop antibodies to hCG, which can lead to testosterone unresponsiveness (28,97,98).

rFSH has been reported to have a better safety and quality profile than its urinary counterparts. In general, rFSH preparations are purer than urine-derived FSH, and the inclusion of mass and vial filling has virtually eliminated batch-to-batch variation and enabled accurate dosing. The most common FSH preparations are recombinant, administered subcutaneously two or three times a week for 3-6 months at doses ranging from 75 to 300 IU (95). Long-acting FSH preparations have also been developed in recent years. Corifollitrophin alpha, a long-acting FSH analogue, needs to be administered every two weeks. Although it has been proven effective, FSH analogues are not commonly used (39).

There is consistent evidence that recombinant rFSH/hCG combination therapy is significantly more effective than hCG alone in both inducing spermatogenesis and increasing testicular volume (69). There is also some evidence that pre-treatment with rFSH followed by combination with hCG or GnRH is even more effective in optimizing Sertoli cell maturation and inducing spermatogenesis in extremely small testes (< 4 mL) (28,38,66). This treatment led to a significant increase in TV (bi-testicular volume: from 5 ± 5 to 34 ± 3 mL) and to the induction of spermatogenesis in 91% of the patients (93). Although hCG alone can increase testicular volume, combined treatment with hCG and FSH has been shown to result in a better response in terms of final testicular size (53). In a meta-analysis study conducted by Alexander et al. (99) in 2023, which included 103 studies with a mean age of less than 25 years, gonadotropin therapy was found to increase testicular volume, penile size, testosterone levels, and spermatogenesis success. The success rate was 86% (82-91%) in patients who received hCG + FSH therapy and 50% (25-56%) in patients who received hCG monotherapy. However, it was emphasized that the treatment options, doses, durations, and results were heterogeneous, and therefore new randomized control studies are needed. Rastrelli et al. (69) conducted a meta-analysis and found that patients who received hCG monotherapy had a significantly lower sperm count compared to those who received hCG + FSH treatment (0.47 million/mL vs 11.57 million/mL, respectively, $p < 0.001$). Various factors affecting the fertility success of physiological induction are summarized in Table 5 (12,87,99).

It is important to note that the physiological pubertal induction protocol has several significant disadvantages, including the requirement for five injections each week, consisting of two hCG injections and three FSH injections. Moreover, acquiring the essential medications may pose challenges contingent upon the economic circumstances prevailing in the country (especially hCG), and the cost is higher compared to traditional parenteral TRT. Moreover, it remains unclear whether a physiological protocol for inducing puberty should be applied to individuals in the pubertal age group. The use of physiological pubertal induction therapy is limited due to its high cost and impractical lifelong use. Although there is no strong evidence to support switching to parenteral TRT after completing physiological pubertal induction therapy, it is reported that TRT can be used once physiological induction therapy is completed (6-24 months) until fertility is desired. However, it is recommended to perform a spermiogram before switching to TRT treatment. If there is enough sperm in the ejaculate, it is advisable to consider sperm cryopreservation (banking), especially in cases of severe oligospermia, to improve future fertility. In cases of azoospermia, individuals may be classified as 'poor responders' to gonadotropin stimulation. In such cases, a testicular sperm extraction procedure may be considered. It is unclear whether spermatogenesis will begin more quickly with repeated physiological induction therapy after TRT treatment. Therefore, a spermiogram should be performed before the transition (38).

Table 5. Factors affecting fertility success during physiological pubertal induction therapy

	Factors affecting success
- History of bilateral undescended testis?	Yes
- Bilateral undescended testis operation time?	> 12 month
- Dysgenetic condition of the testes?	Yes
- Etiology of HypoH? Is it congenital or acquired?	Congenital?
- Prior exposure to androgens?	Controversial
- Is the minipubertal period physiologically mimicked in permanent hypogonadotropic hypogonadism?	No long-term data
- Testicular volumes before treatment?	< 4 mL
- Basal inhibin B levels?	< 10 pg/mL

HypoH: hypogonadotropic hypogonadism

For cases with acquired permanent HH where pubertal arrest (TV >4 mL) has occurred or minipuberty has been experienced, fertility probabilities are greatly increased with the application of a physiological induction protocol in advanced ages, even if the physiological induction protocol was not applied during the pubertal period (53).

Should Physiological Induction Be Performed During the Minipuberty or Prepubertal Period?

FSH is necessary for the development of the Sertoli cell population. Following birth, Sertoli cells proliferate under the control of FSH during the first few months of life and in early puberty. The number of Sertoli cells is directly related to sperm production capacity. Each of these somatic cells can only support a limited number of developing spermatogenic cells. In line with these findings, it has been suggested that male patients who have not experienced mini-pubertal periods as a result of CHH have a poor response to pulsatile GnRH in terms of testicular growth and spermatogenesis (28,32).

Infants with micropenis associated with HH require TRT to increase the length of the penis. However, gonadotropin therapy with recombinant human FSH (rhFSH) and rhLH can also be used to enhance testicular enlargement before orchiopexy and correct micropenis (28,85,86,87,100). In recent years, it has been proposed to use physiological mimicry of the mini-puberty process with rhFSH and rhLH to increase the fertility potential of patients with CHH in adulthood. It is important to note that this approach is still being researched and is not yet widely used. In small groups of patients, different physiological induction protocols (continuous pump infusion or intermittent subcutaneous injection) have been used during the postnatal period of 0.7-6 months (mini-puberty), resulting in increases in testicular volume, penile length, testosterone, and inhibin B levels (85,86,87,101). However, these trials, which physiologically mimicked mini-puberty, did not provide information on adult fertility outcomes (85,86,87,101).

In an experimental study conducted in 2005, it was shown that administering FSH for four months before induction of puberty increased testicular volume and inhibin B levels, and also increased the number of Sertoli cells and type A spermatogonia (102). Following this experimental study, in 2007, Raivio et al. (103) proposed a new treatment for prepubertal boys with congenital and acquired HH, using rhFSH to increase sperm production by inducing the proliferation of immature Sertoli cells before hCG treatment. This study included 14 prepubertal male patients with different diagnoses: two patients with idiopathic HH, two with KS, four with idiopathic panhypopituitarism, and six with organic panhypopituitarism. The patients were aged between 9.9 and 17 years and had a testicular volume of less than 3 mL. The patients underwent rhFSH priming (1.5 IU/kg/dose, 3 times a week) for two months-2.8 years. The study found that there was a significant increase in testicular volume and inhibin B levels (0.9 ± 0.6 mL to 1.8 ± 1.1 mL, $p < 0.005$ and 27 ± 14 to 80 ± 57 pg/mL, $p < 0.01$, respectively). Spermatogenesis was successful in 6 out of 7 boys (86%) who provided semen samples, with a maximum sperm count ranging from 2.9 to 92 million/mL (median 8.5 million/mL). It was emphasized that the proliferation of the germ cell pool is important for fertility success and that FSH priming is necessary before hCG treatment (103). They emphasized that poor inhibin B responses in three patients who did not have postnatal hypothalamic-pituitary axis activation (indicating that they did not experience mini-puberty) would negatively affect future fertility success. One of the major limitations of this study is that patients were not classified by etiology, presence or absence of cryptorchidism, and the duration of FSH was not standardized. To achieve better outcomes in future studies, it is recommended to classify patients according to etiology and standardize FSH duration. The high fertility success observed in this study may be related to the inclusion of patients diagnosed with acquired HH with pubertal arrest.

Following the demonstration of the effect of FSH priming on fertility success in the experimental study by Pitteloud and Dwyer et al. (66) in 2005 (102), an open randomized controlled trial was conducted by the same authors in 2013 in 13 male patients with CHH and no history of undescended testicles. Seven patients received rhFSH (75-150 IU SC QD) for four months, followed by treatment with GnRH and the other group ($n = 6$) received only GnRH from the start of treatment. At the 24th month of treatment, testicular volume, sperm count, and fertility success were significantly higher in the FSH-primed group compared with the non-FSH-primed group (testicular volumes; 9.3 ± 1.7 mL and 6.6 ± 1.3 mL; sperm counts; 5.8 ± 2.3 and $2.6 \pm 1.5/10^6$ mL, fertility success, 100% and 66% respectively). In this study, although the mini-puberty period was not experienced in the FSH priming group, all cases were considered to be fertile (66). The results of this study suggest that, contrary to the study by Raivio et al. (103), the physiological mimicry of the minipubertal period is not mandatory (66). Randomized studies with large patient populations are needed to reach firmer conclusions in this area.

Although these two studies (66,103) make a valuable contribution to the literature, the most important drawbacks are the small number of cases included in the groups

studied. In addition, the heterogeneity of the diagnoses in the study group of Raivio et al. (103) does not provide strong evidence for the mimicry of the mini-pubertal period. The physiological induction protocols that have been used in the mini-pubertal period have been used as non-standardized protocols in isolated cases or small groups of patients, and there is a lack of data on the fertility outcomes. However, published studies show that combined gonadotropin therapy has a more beneficial effect than parenteral TRT on testicular (Sertoli cell proliferation and seminiferous tubule growth) and genital development (increase in TV and penile length) in male patients with CHH during the mini-pubertal period (85,86,87,101). Despite the beneficial effects of gonadotropin therapy, there is still a need for strong evidence to support the physiological mimicry of the mini-pubertal period. Existing studies suggest the need for robust randomized and controlled trials with large patient populations in which: (i) the groups are diagnostically homogeneous; (ii) the mini-pubertal period is mimicked or not with physiologically standardized protocols; and (iii) the cases with or without undescended testes are grouped. Although there are different physiological induction protocols for gonadotropins in the mini-pubertal and pubertal periods in boys, there are no data on physiological induction in the mini-pubertal period in girls with CHH. In contrast, there are gonadotropin protocols for ovulation induction in adult female patients with CHH (12).

Physiological Pubertal Induction in Male Patients

In male patients, the physiological pubertal induction protocol can be started from the age of 12 years in cases with confirmed congenital HH. In cases of unconfirmed diagnosis, it should be started after the necessary differential diagnoses have been made (11,38).

Normal levels of both gonadotropins are necessary for appropriate spermatogenesis induction during puberty (53). The optimal treatment regimen should be used when a patient has inadequate pubertal development and a testicular size of less than 4 mL. Treatment of prepubertal patients (testicular volume < 4 mL) initially with rhFSH for four months, to maximize the Sertoli cell pool, followed by combination treatment with rhFSH and hCG has been suggested as the most favorable strategy for future fertility (93,104). FSH maximizes Sertoli and germ cell counts, and increases seminiferous tubule growth (7). Recombinant hCG, which shares a receptor with LH, increases serum testosterone levels, both of which lead to normal spermatogenesis (66). For patients experiencing spontaneous onset of puberty or pubertal arrest (due to any etiological reason), with a testicular volume of 4 mL or more, hCG monotherapy or hCG combined therapy with FSH can be initiated as the primary treatment option (53,93).

Rohayem et al. (93) studied a relatively large group (n = 34) of adolescents with delayed puberty, with the majority of them having no signs of puberty. The adolescents were treated with low doses of hCG (250-500 IU twice a week) with gradual increases of 250-500 IU every six months, and when the target pubertal level of serum testosterone (5.2 nmol/L = 150 ng/dL) was reached, rFSH was introduced (93). Typically, it is advised to provide hCG at a dosage of 500-2500 IU per dose, 2-3 times per week, towards the conclusion of the treatment (12). The literature states that the recommended dosage ranges from 3000 to 10000 IU each dose, administered 2 to 3 times per week (105). The initial dosage of FSH is typically 75-150 IU (or 1 IU/kg/dose) administered every other day (or 3 times per week) (106). To attain a serum FSH concentration within the physiological range of 1-7 IU/L, the dosage should be increased if deemed required (93). The physiological induction protocol in male patients was revised according to the recommendations of Rohayem et al. (93) (German Adolescent Hypogonadotropic Hypogonadism Study Group). Figures 3 and 4 summarize the general recommendation for physiological pubertal induction in male patients (53,93).

In addition to the assessment of the development of the secondary sex characteristics, serum levels of FSH, inhibin B, total testosterone, and hemoglobin should be monitored at 3-month intervals to assess safety and efficacy (12). The dose of hCG should be adjusted according to testosterone levels, while the dose of FSH is typically modified based on the clinical signs and FSH levels (Figure 4) (53,66,93). The half-life of hCG is approximately 36 hours. Therefore, total testosterone and estradiol concentrations obtained before the subsequent injection are the most informative indicators for ensuring that the target testosterone concentration is being maintained. Normal serum testosterone concentrations may not be achieved due to poor adherence or, rarely, the development of antibodies (106).

For patients with CHH who have GnRH deficiency but normal pituitary function, pulsatile GnRH treatment may be a viable option for both sexes (12). The most physiological approach is to use GnRH infused in a pulsatile fashion, with pulse intervals of 90-120 minutes (53). Pulsatile GnRH treatment stimulating the release of endogenous FSH and LH is effective in normalizing the gonadal axis of the majority of the patients with HH (except GnRH receptor defect) (73). Even in patients with combined pituitary hormone deficiencies, in the presence of a pituitary reserve, the pituitary-testis axis function is restored in 60% of all cases, while displaying no association with the pituitary

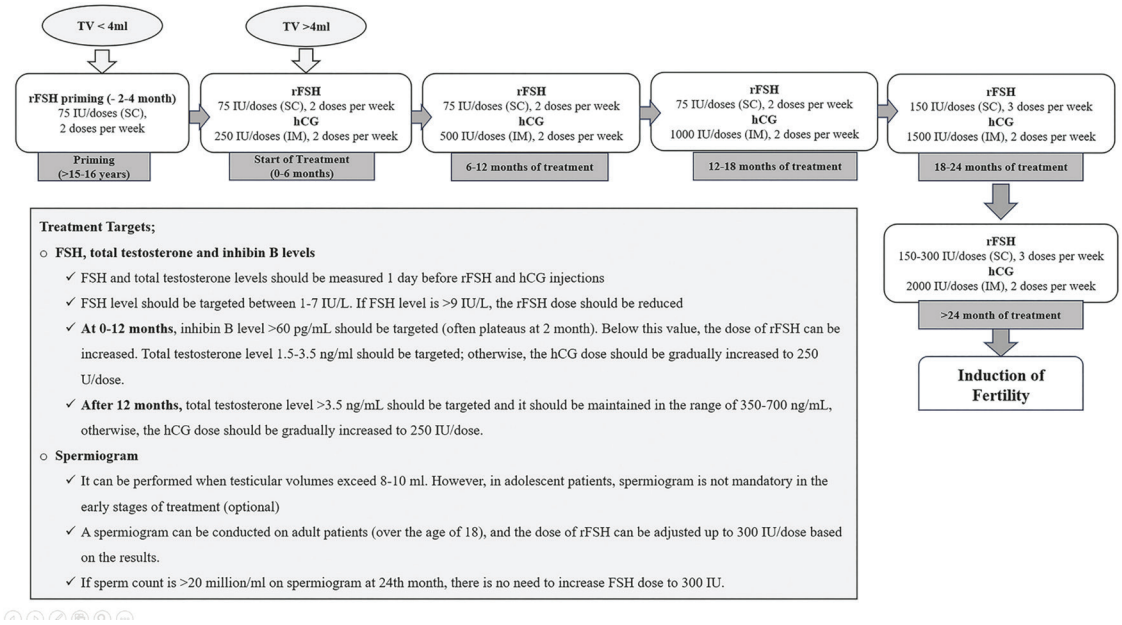

Figure 4. Graphic timeline of recombinant FSH and hCG hormone treatment plan, assessments, and treatment goals

FSH: follicular stimulating hormone, hCG: human chorionic gonadotropin hormone, rFSH: recombinant FSH, IM: intramuscular

height or integrity. Liu et al. (107) found that pulsatile GnRH therapy for two years in adolescents with the complete form of CHH does not significantly enhance testicular growth, accelerate the onset of sperm production, or increase sperm output compared to hCG/human menopausal gonadotropin therapy. While there are several different regimens, it is recommended to start GnRH treatment with 5-25 ng/kg per pulse administrated at 90-120-minute intervals with an increase of 2 ng every month, targeting testosterone levels in the mid-normal adult ranges (13,108).

A review by Young et al. (12), evaluating the efficacy of GnRH (n = 11 trials) and combined gonadotropin therapy (n = 33 trials) among 1118 patients, demonstrated that the median testicular volume increased from 3.4 mL to 9.8 mL and median sperm count increased from 7.59 million/mL to 15.3 million/mL. Persistent azoospermia was found in 17% (38/219) of patients treated with GnRH infusion, compared to 21% (190/899) of patients treated with combined gonadotropins (FSH + hCG).

Physiological Protocols for Inducing Puberty in Female Subjects

The overall goal of sex hormone replacement therapy in girls with hypogonadism is to establish an age-appropriate endocrine milieu resulting in normal growth, bone mass accrual, uterine growth and maturation, and development of secondary sexual characteristics and cognitive functions, at a tempo consistent with their peer group. The hormone replacement therapy process in adolescent girls consists of three main stages. It has been suggested that low levels of estrogen in healthy pre-pubertal girls may promote the maturation of the bones and growth. Therefore, it is recommended to start with very low doses of estrogen therapy in the early stages, which will not cause breast development but will contribute to growth and bone maturation. In the second stage, low doses of estrogen therapy should be initiated to ensure the physiologic pubertal developmental stages and the development of secondary sex characteristics, and the dose should be increased in certain intervals. In the last stage (2-3 years of treatment), when the final estrogen dose is reached, progesterone should be added to the treatment to ensure menarche in patients who have completed pubertal development. This treatment should be continued until the age of menopause (56).

Determining the optimal route, drug, dose, and timing of estrogen replacement treatment for girls with hypogonadism is an active area of research. There is currently no agreement in the literature regarding the most suitable approach. Treatment should be individualized (56). The most common and preferred form of estrogen replacement is 17-β-estradiol. Oral 17-β-estradiol products (ethinyl estradiol, conjugated equine estrogen) can be initiated as biphasic or triphasic sequential hormone replacement regimens combined with progestins. The triphasic regimens are useful in providing lower estrogen doses during the treatment-free week of the biphasic regimens, hence effectively controlling vasomotor symptoms, which can be particularly valuable in those with established diagnosis and

older ages (109). However, oral forms have been associated with a higher risk of thromboembolism than transdermal products. Therefore, transdermal estrogen therapy should be the first choice for pubertal induction because it bypasses hepatic metabolism and has been shown to result in more stable serum estradiol concentrations with no reduction in IGF-1 concentrations compared to oral forms (15,110,111). However, no significant differences in body composition, height, or bone mineralization have been found in studies directly comparing oral and transdermal estrogen (15). Table 2 shows the dosage and route of administration of different estrogen preparations for pubertal induction. Although different protocols are available, the transdermal estrogen protocol used in this review is based on the protocol published by Ankarberg-Lindgren et al. (12,54,55,56) in 2001 and 2014. The transdermal estrogen protocol is summarized in Figure 5.

Progestins are initiated for withdrawal bleeding after 2-3 years of estrogen treatment or when a significant breakthrough bleeding occurs under estrogen treatment (111). Micronized crystalline progesterone (100-200 mg/day) or medroxyprogesterone acetate (5-10 mg/day) are preferred and are administrated for 5-10 days each month to prevent endometrial hypertrophy (Figure 6) (56,110). Gonadotropin or GnRH infusion therapy protocols are not commonly used for pubertal induction in adolescent girls. In contrast, gonadotropin treatment protocols are used for the induction of ovulation in female patients expected to be fertile in adulthood (12).

Conclusion

Hormone treatment is essential in hypogonadism as sex hormone deficiency can lead to several complications, including osteoporosis, changes in body composition,

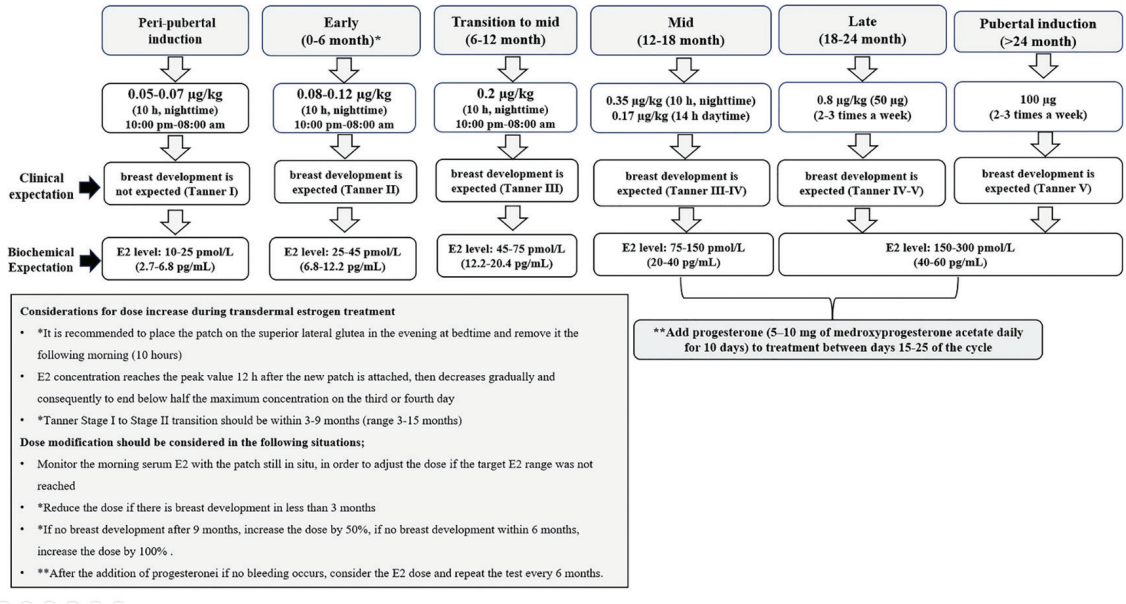

Figure 5. Pubertal induction protocol with transdermal estrogen therapy in girls

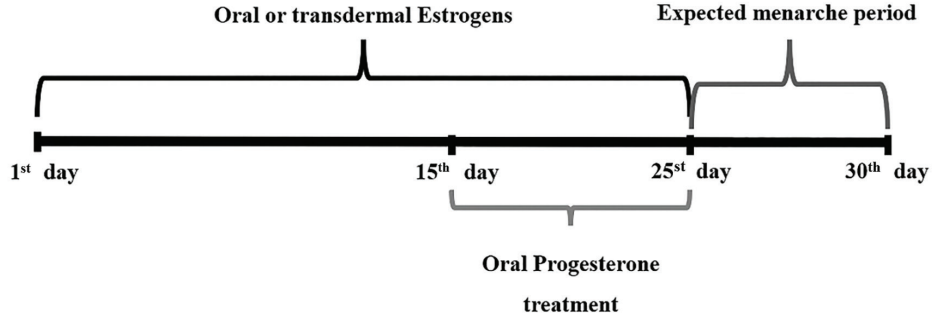

Figure 6. Monthly administration of estrogen and progesterone after the induction of puberty in an adolescent girl

metabolic abnormalities, cardiovascular risks, and mood disorders. The aim is to help achieve physiological and psychological adolescent maturation for the normal development of secondary sexual characteristics, uterine/testicular growth, bone mass, and growth spurt. Treatment strategies should be individualized with meticulous dose titrations to balance the expectations on pubertal progression and expected adult height.

In recent years, physiological pubertal induction protocols (recombinant gonadotropins or GnRH analog) have been recommended to increase fertility success in cases of persistent HH. The protocol allows induction of spermatogenesis in the majority (80-90%) of cases after two years. The treatment is generally well accepted and tolerated by patients.

However, due to the inconvenience of application, difficulties in obtaining drugs, and the lack of strong evidence that TRT decreases fertility success, the use of physiological induction protocols from mini-puberty and adolescence is still controversial. Randomized, case-controlled studies are needed to support the use of physiological induction protocols in mini-pubertal and adolescent populations.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayhan Abacı, Concept: Ayhan Abacı, Design: Ayhan Abacı, Data Collection or Processing: Ayhan Abacı, Özge Besci, Analysis or Interpretation: Ayhan Abacı, Literature Search: Ayhan Abacı, Özge Besci, Writing: Ayhan Abacı, Özge Besci.

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References

1. Kaplowitz PB. Delayed puberty. Pediatr Rev. 2010;31:189-195.
2. Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, Prevot V, Raivio T, Tena-Sempere M, Quinton R, Young J. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11:547-564. Epub 2015 Jul 21
3. Seppä S, Kuiri-Hänninen T, Holopainen E, Voutilainen R. Management of Endocrine Disease: Diagnosis and management of primary amenorrhea and female delayed puberty. Eur J Endocrinol. 2021;184:225-242.
4. Villanueva C, Argente J. Pathology or normal variant: what constitutes a delay in puberty? Horm Res Paediatr. 2014;82:213-221. Epub 2014 Jul 7
5. Prévot V, Tena-Sempere M, Pitteloud N. New Horizons: Gonadotropin-Releasing Hormone and Cognition. J Clin Endocrinol Metab. 2023;108:2747-2758.
6. Lucaccioni L, Trevisani V, Boncompagni A, Marrozzini L, Berardi A, Iughetti L. Minipuberty: Looking Back to Understand Moving Forward. Front Pediatr. 2020;8:612235.
7. S Swee DS, Quinton R. Congenital Hypogonadotrophic Hypogonadism: Minipuberty and the Case for Neonatal Diagnosis. Front Endocrinol (Lausanne). 2019;10:97.
8. Edelsztein NY, Grinspon RP, Schteingart HF, Rey RA. Anti-Müllerian hormone as a marker of steroid and gonadotropin action in the testis of children and adolescents with disorders of the gonadal axis. Int J Pediatr Endocrinol. 2016;2016:20. Epub 2016 Oct 28
9. Howard SR, Dunkel L. Delayed Puberty-Phenotypic Diversity, Molecular Genetic Mechanisms, and Recent Discoveries. Endocr Rev. 2019;40:1285-1317.
10. Farello G, Altieri C, Cutini M, Pozzobon G, Verrotti A. Review of the Literature on Current Changes in the Timing of Pubertal Development and the Incomplete Forms of Early Puberty. Front Pediatr. 2019;7:147.
11. Rosenfield RL, Lipton RB, Drum ML. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. Pediatrics. 2009;123:84-88.
12. Young J, Xu C, Papadakis GE, Acierno JS, Maione L, Hietamäki J, Raivio T, Pitteloud N. Clinical Management of Congenital Hypogonadotropic Hypogonadism. Endocr Rev. 2019;40:669-710.
13. Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic hypogonadism? Clin Endocrinol (Oxf). 2010;72:731-737. Epub 2009 Nov 11
14. Palmert MR, Dunkel L. Clinical practice. Delayed puberty. N Engl J Med. 2012;366:443-453.
15. Harrington J, Palmert MR. An Approach to the Patient With Delayed Puberty. J Clin Endocrinol Metab. 2022;107:1739-1750.
16. Traggiai C, Stanhope R. Delayed puberty. Best Pract Res Clin Endocrinol Metab. 2002;16:139-151.
17. Palmert MR, Chan YM, Dunkel L. Puberty and Its Disorders in the Male In: Sperling MA, Majzoub JA, Menon RK, Stratakis CA (eds). Sperling Pediatric Endocrinology. Philadelphia Elsevier. 2020:661-694.
18. Mohanraj S, Prasad HK. Delayed Puberty. Indian J Pediatr. 2023;90:590-597.
19. Bakhtiani P, Geffner M. Delayed Puberty. Pediatr Rev. 2022;43:426-435.
20. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. J Clin Endocrinol Metab. 2002;87:1613-1620.
21. Kohva E, Huopio H, Hero M, Miettinen PJ, Vaaralahti K, Sidoroff V, Toppari J, Raivio T. Recombinant Human FSH Treatment Outcomes in Five Boys With Severe Congenital Hypogonadotropic Hypogonadism. J Endocr Soc. 2018;2:1345-1356.
22. Laitinen EM, Vaaralahti K, Tommiska J, Eklund E, Tervaniemi M, Valanne L, Raivio T. Incidence, phenotypic features and molecular genetics of Kallmann syndrome in Finland. Orphanet J Rare Dis. 2011;6:41.
23. Filippi G. Klinefelter's syndrome in Sardinia. Clinical report of 265 hypogonadic males detected at the time of military check-up. Clin Genet. 1986;30:276-284.
24. Topaloğlu AK. Update on the Genetics of Idiopathic Hypogonadotropic Hypogonadism. J Clin Res Pediatr Endocrinol. 2017;9(Suppl 2):113-122. Epub 2017 Dec 27
25. Stamou MI, Georgopoulos NA. Kallmann syndrome: phenotype and genotype of hypogonadotropic hypogonadism. Metabolism. 2018;86:124-134. Epub 2017 Nov 3
26. Hietamäki J, Hero M, Holopainen E, Känsäkoski J, Vaaralahti K, Iivonen AP, Miettinen PJ, Raivio T. GnRH receptor gene mutations

in adolescents and young adults presenting with signs of partial gonadotropin deficiency. PLoS One. 2017;12:e0188750.

27. Liu Y, Zhi X. Advances in Genetic Diagnosis of Kallmann Syndrome and Genetic Interruption. Reprod Sci. 2022;29:1697-1709. Epub 2021 Jul 6
28. Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, Hughes VA, Cole LW, Pearce SH, Lee H, Boepple P, Crowley WF Jr, Pitteloud N. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007;357:863-873.
29. Dwyer AA, Raivio T, Pitteloud N. Management Of Endocrine Disease: Reversible hypogonadotropic hypogonadism. Eur J Endocrinol. 2016;174:267-274. Epub 2016 Jan 20
30. Salenave S, Trabado S, Maione L, Brailly-Tabard S, Young J. Male acquired hypogonadotropic hypogonadism: diagnosis and treatment. Ann Endocrinol (Paris). 2012;73:141-146. Epub 2012 Apr 25
31. Zhu J, Liu E, Feld A, Jonsdottir-Lewis E, Shirey A, Feldman HA, Astley CM, Chan YM. Approaches to Identify Factors Associated with Pubertal Timing in Self-Limited Delayed Puberty. Horm Res Paediatr. 2023;96:267-277. Epub 2022 Aug 25
32. Raivio T, Miettinen PJ. Constitutional delay of puberty versus congenital hypogonadotropic hypogonadism: Genetics, management and updates. Best Pract Res Clin Endocrinol Metab. 2019;33:101316. Epub 2019 Sep 5
33. Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. J Clin Endocrinol Metab. 2012;97:3056-3067. Epub 2012 Jun 20
34. Dwyer AA, Chavan NR, Lewkowitz-Shpuntoff H, Plummer L, Hayes FJ, Seminara SB, Crowley WF, Pitteloud N, Balasubramanian R. Functional Hypogonadotropic Hypogonadism in Men: Underlying Neuroendocrine Mechanisms and Natural History. J Clin Endocrinol Metab. 2019;104:3403-3414.
35. Viswanathan V, Eugster EA. Etiology and treatment of hypogonadism in adolescents. Pediatr Clin North Am. 2011;58:1181-1200.
36. Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, Murad MH, Santoro NF, Warren MP. Functional Hypothalamic Amenorrhea: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2017;102:1413-1439.
37. Nieschlag E, Behre HM, Bouchard P, Corrales JJ, Jones TH, Stalla GK, Webb SM, Wu FC. Testosterone replacement therapy: current trends and future directions. Hum Reprod Update. 2004;10:409-419. Epub 2004 Aug 5
38. Nordenström A, Ahmed SF, van den Akker E, Blair J, Bonomi M, Brachet C, Broersen LHA, Claahsen-van der Grinten HL, Dessens AB, Gawlik A, Gravholt CH, Juul A, Krausz C, Raivio T, Smyth A, Touraine P, Vitali D, Dekkers OM. Pubertal induction and transition to adult sex hormone replacement in patients with congenital pituitary or gonadal reproductive hormone deficiency: an Endo-ERN clinical practice guideline. Eur J Endocrinol. 2022;186:9-49.
39. Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. J Clin Endocrinol Metab. 2009;94:801-808. Epub 2008 Dec 9
40. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16:231-245. Epub 2009 Nov 24
41. Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic hypogonadism revisited. Clinics (Sao Paulo). 2013;68(Suppl 1):81-88.
42. Lambert AS, Bouvattier C. Puberty induction with recombinant gonadotropin: What impact on future fertility? Ann Endocrinol (Paris). 2022;83:159-163. Epub 2022 Apr 15
43. Corona G, Goulis DG, Liu PY. The biochemical confirmation of adult male hypogonadism: Global perspectives from the International Society of Andrology. Clin Endocrinol (Oxf). 2023;99:398-400. Epub 2023 Jun 9
44. Jayasena CN, de Silva NL, O'Reilly MW, MacKenzie F, Marrington R, Jones H, Livingston M, Downie P, Hackett G, Ramachandran S, Tomlinson J, David J, Boot C, Patel M, Tarling J, Wu F, Quinton R. Standardising the biochemical confirmation of adult male hypogonadism: A joint position statement by the Society for Endocrinology and Association of Clinical Biochemistry and Laboratory Medicine. Clin Endocrinol (Oxf). 2024;101:531-534. Epub 2023 Jul 1
45. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. J Clin Endocrinol Metab. 2007;92:1424-1429. Epub 2007 Feb 6
46. Chellakooty M, Schmidt IM, Haavisto AM, Boisen KA, Damgaard IN, Mau C, Petersen JH, Juul A, Skakkebaek NE, Main KM. Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. J Clin Endocrinol Metab. 2003;88:3515-3520.
47. Koysombat K, Dhillo WS, Abbara A. Assessing hypothalamic pituitary gonadal function in reproductive disorders. Clin Sci (Lond). 2023;137:863-879.
48. Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, Rouleau S, Lahlou N. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. J Clin Endocrinol Metab. 2010;95:5225-5232. Epub 2010 Sep 8
49. Klein KO, Phillips SA. Review of Hormone Replacement Therapy in Girls and Adolescents with Hypogonadism. J Pediatr Adolesc Gynecol. 2019;32:460-468. Epub 2019 May 3
50. Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. Andrology. 2015;3:882-887. Epub 2015 Aug 12
51. Binder G, Schweizer R, Blumenstock G, Braun R. Inhibin B plus LH vs GnRH agonist test for distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism in boys. Clin Endocrinol (Oxf). 2015;82:100-105. Epub 2014 Oct 23
52. Grinspon RP, Rey RA. Anti-müllerian hormone and sertoli cell function in paediatric male hypogonadism. Horm Res Paediatr. 2010;73:81-92. Epub 2010 Feb 9
53. Alenazi MS, Alqahtani AM, Ahmad MM, Almalki EM, AlMutair A, Almalki M. Puberty Induction in Adolescent Males: Current Practice. Cureus. 2022;14:e23864.
54. Ankarberg-Lindgren C, Kriström B, Norjavaara E. Physiological estrogen replacement therapy for puberty induction in girls: a clinical observational study. Horm Res Paediatr. 2014;81:239-244. Epub 2014 Feb 4
55. Ankarberg-Lindgren C, Elfving M, Wikland KA, Norjavaara E. Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. J Clin Endocrinol Metab. 2001;86:3039-3044.
56. Norjavaara E, Ankarberg-Lindgren C, Kriström B. Sex Steroid Replacement Therapy in Female Hypogonadism from Childhood to Young Adulthood. Endocr Dev. 2016;29:198-213. Epub 2015 Dec 17
57. Howell S, Shalet S. Testosterone deficiency and replacement. Horm Res. 2001;56(Suppl 1):86-92.
58. Handelsman DJ, Conway AJ, Boylan LM. Pharmacokinetics and pharmacodynamics of testosterone pellets in man. J Clin Endocrinol Metab. 1990;71:216-222.

59. Klein DA, Emerick JE, Sylvester JE, Vogt KS. Disorders of Puberty: An Approach to Diagnosis and Management. Am Fam Physician. 2017;96:590-599.

60. Pozo J, Argente J. Ascertainment and treatment of delayed puberty. Horm Res. 2003;60(Suppl 3):35-48.

61. Mieszczak J, Houk CP, Lee PA. Management of Disordered Puberty. Brook's Clinical Pediatric Endocrinology, 2009:239-249.

62. Varimo T, Huopio H, Kariola L, Tenhola S, Voutilainen R, Toppari J, Toiviainen-Salo S, Hämäläinen E, Pulkkinen MA, Lääperi M, Tarkkanen A, Vaaralahti K, Miettinen PJ, Hero M, Raivio T. Letrozole versus testosterone for promotion of endogenous puberty in boys with constitutional delay of growth and puberty: a randomised controlled phase 3 trial. Lancet Child Adolesc Health. 2019;3:109-120. Epub 2019 Jan 4

63. Mauras N, Ross J, Mericq V. Management of Growth Disorders in Puberty: GH, GnRHa, and Aromatase Inhibitors: A Clinical Review. Endocr Rev. 2023;44:1-13.

64. Stancampiano MR, Lucas-Herald AK, Russo G, Rogol AD, Ahmed SF. Testosterone Therapy in Adolescent Boys: The Need for a Structured Approach. Horm Res Paediatr. 2019;92:215-228. Epub 2019 Dec 18

65. Wit JM, Balen HV, Kamp GA, Oostdijk W. Benefit of postponing normal puberty for improving final height. Eur J Endocrinol. 2004;151(Suppl 1):41-45.

66. Dwyer AA, Sykiotis GP, Hayes FJ, Boepple PA, Lee H, Loughlin KR, Dym M, Sluss PM, Crowley WF Jr, Pitteloud N. Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2013;98:1790-1795. Epub 2013 Sep 13

67. Majzoub A, Sabanegh E Jr. Testosterone replacement in the infertile man. Transl Androl Urol. 2016;5:859-865.

68. World Health Organization Task Force on Methods for the Regulation of Male Fertility. Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril. 1996;65:821-829.

69. Rastrelli G, Corona G, Mannucci E, Maggi M. Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. Andrology. 2014;2:794-808. Epub 2014 Oct 1

70. Sidhoum VF, Chan YM, Lippincott MF, Balasubramanian R, Quinton R, Plummer L, Dwyer A, Pitteloud N, Hayes FJ, Hall JE, Martin KA, Boepple PA, Seminara SB. Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system. J Clin Endocrinol Metab. 2014;99:861-870. Epub 2013 Jan 1

71. Chioma L, Cappa M. Hypogonadism in Male Infants and Adolescents: New Androgen Formulations. Horm Res Paediatr. 2023;96:581-589. Epub 2021 Dec 16

72. Shoskes JJ, Wilson MK, Spinner ML. Pharmacology of testosterone replacement therapy preparations. Transl Androl Urol. 2016;5:834-843.

73. Nieschlag E, Behre HM, Nieschlag S. Testosterone: Action, Deficiency, Substitution: Cambridge University Press, 2012.

74. Minnemann T, Schubert M, Freude S, Hübler D, Gouni-Berthold I, Schumann C, Christoph A, Oettel M, Ernst M, Mellinger U, Krone W, Jockenhövel F. Comparison of a new long-acting testosterone undecanoate formulation vs testosterone enanthate for intramuscular androgen therapy in male hypogonadism. J Endocrinol Invest. 2008;31:718-723.

75. Santhakumar A, Miller M, Quinton R. Pubertal induction in adult males with isolated hypogonadotropic hypogonadism using long-acting intramuscular testosterone undecanoate 1-g depot (Nebido). Clin Endocrinol (Oxf). 2014;80:155-157. Epub 2013 May 20

76. Dattani MT, Brook CGD. Brook's Clinical Pediatric Endocrinology: Wiley, 2019.

77. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM; Task Force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;95:2536-2559.

78. Stastny K, Putecova K, Leva L, Franek M, Dvorak P, Faldyna M. Profiling of Metabolomic Changes in Plasma and Urine of Pigs Caused by Illegal Administration of Testosterone Esters. Metabolites. 2020;10:307.

79. Gurayah AA, Dullea A, Weber A, Masterson JM, Khodamoradi K, Mohamed AI, Ramasamy R. Long vs Short Acting Testosterone Treatments: A Look at the Risks. Urology. 2023;172:5-12. Epub 2022 Nov 28

80. Pastuszak AW, Gittelman M, Tursi JP, Jaffe JS, Schofield D, Miner MM. Pharmacokinetics of testosterone therapies in relation to diurnal variation of serum testosterone levels as men age. Andrology. 2022;10:209-222. Epub 2021 Oct 8

81. Nieschlag E, Behre HM, Nieschlag S. Andrology: Male Reproductive Health and Dysfunction: Springer Berlin Heidelberg, 2010.

82. Penson D, Krishnaswami S, Jules A, McPheeters ML. Effectiveness of hormonal and surgical therapies for cryptorchidism: a systematic review. Pediatrics. 2013;131:1897-1907. Epub 2013 May 20

83. Dunkel L, Taskinen S, Hovatta O, Tilly JL, Wikström S. Germ cell apoptosis after treatment of cryptorchidism with human chorionic gonadotropin is associated with impaired reproductive function in the adult. J Clin Invest. 1997;100:2341-2346.

84. Hjertkvist M, Läckgren G, Plöen L, Bergh A. Does HCG treatment induce inflammation-like changes in undescended testes in boys? J Pediatr Surg. 1993;28:254-258.

85. Main KM, Schmidt IM, Toppari J, Skakkebaek NE. Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH. Eur J Endocrinol. 2002;146:75-79.

86. Stoupa A, Samara-Boustani D, Flechtner I, Pinto G, Jourdon I, González-Briceño L, Bidet M, Laborde K, Chevenne D, Millischer AE, Lottmann H, Blanc T, Aigrain Y, Polak M, Beltrand J. Efficacy and Safety of Continuous Subcutaneous Infusion of Recombinant Human Gonadotropins for Congenital Micropenis during Early Infancy. Horm Res Paediatr. 2017;87:103-110. Epub 2017 Jan 12

87. Bougnères P, François M, Pantalone L, Rodrigue D, Bouvattier C, Demesteere E, Roger D, Lahlou N. Effects of an early postnatal treatment of hypogonadotropic hypogonadism with a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone. J Clin Endocrinol Metab. 2008;93:2202-2205. Epub 2008 Apr 1

88. Grinspon RP, Urrutia M, Rey RA. Male Central Hypogonadism in Paediatrics - the Relevance of Follicle-stimulating Hormone and Sertoli Cell Markers. Eur Endocrinol. 2018;14:67-71. Epub 2018 Sep 10

89. Hatipoğlu N, Kurtoğlu S. Micropenis: etiology, diagnosis and treatment approaches. J Clin Res Pediatr Endocrinol. 2013;5:217-223.

90. Tsang S. When size matters: a clinical review of pathological micropenis. J Pediatr Health Care. 2010;24:231-240. Epub 2009 Jul 23

91. Kaya C, Bektic J, Radmayr C, Schwentner C, Bartsch G, Oswald J. The efficacy of dihydrotestosterone transdermal gel before primary hypospadias surgery: a prospective, controlled, randomized study. J Urol. 2008;179:684-688.

92. Dwyer AA, Jayasena CN, Quinton R. Congenital hypogonadotropic hypogonadism: implications of absent mini-puberty. Minerva Endocrinol. 2016;41:188-195.

93. Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M; "German Adolescent Hypogonadotropic Hypogonadism Study Group". Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? -a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. Clin Endocrinol (Oxf). 2017;86:75-87. Epub 2016 Sep 7

94. Davidoff AW, Hill MD, Cramer SC, Yang Y, Moore A. Open labeled, uncontrolled pharmacokinetic study of a single intramuscular hCG dose in healthy male volunteers. Int J Clin Pharmacol Ther. 2009;47:516-524.

95. Esteves SC, Achermann APP, Simoni M, Santi D, Casarini L. Male infertility and gonadotropin treatment: What can we learn from real-world data? Best Pract Res Clin Obstet Gynaecol. 2023;86:102310. Epub 2022 Dec 29

96. Cailleux-Bounacer A, Reznik Y, Cauliez B, Menard JF, Duparc C, Kuhn JM. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. Eur J Endocrinol. 2008;159:171-178. Epub 2008 May 21

97. Thau RB, Goldstein M, Yamamoto Y, Burrow GN, Phillips D, Bardin CW. Failure of gonadotropin therapy secondary to chorionic gonadotropin-induced antibodies. J Clin Endocrinol Metab. 1988;66:862-867.

98. Claustrat B, David L, Faure A, Francois R. Development of anti-human chorionic gonadotropin antibodies in patients with hypogonadotropic hypogonadism. A study of four patients. J Clin Endocrinol Metab. 1983;57:1041-1047.

99. Alexander EC, Faruqi D, Farquhar R, Unadkat A, Ng Yin K, Hoskyns R, Varughese R, Howard SR. Gonadotropins for pubertal induction in males with hypogonadotropic hypogonadism: systematic review and meta-analysis. Eur J Endocrinol. 2024;190:1-11.

100. Kohva E, Huopio H, Hietamäki J, Hero M, Miettinen PJ, Raivio T. Treatment of gonadotropin deficiency during the first year of life: long-term observation and outcome in five boys. Hum Reprod. 2019;34:863-871.

101. Papadimitriou DT, Chrysis D, Nyktari G, Zoupanos G, Liakou E, Papadimitriou A, Mastorakos G. Replacement of Male Mini-Puberty. J Endocr Soc. 2019;3:1275-1282.

102. Pitteloud N, Dwyer A, Hayes F, Kumar P, Dym M, Crowley WJ. The role of FSH in human testicular development. Endo 2005 Abstract Book, San Diego 4-7 2005. 2005:1-559. Available from: https://www.medscape.com/viewcollection/4168

103. Raivio T, Wikström AM, Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. Eur J Endocrinol. 2007;156:105-111.

104. Sahib BO, Hussein IH, Alibrahim NT, Mansour AA. Management Outcomes in Males With Hypogonadotropic Hypogonadism Treated With Gonadotropins. Cureus. 2023;15:e35601.

105. Kohn TP, Louis MR, Pickett SM, Lindgren MC, Kohn JR, Pastuszak AW, Lipshultz LI. Age and duration of testosterone therapy predict time to return of sperm count after human chorionic gonadotropin therapy. Fertil Steril. 2017;107:351-357. Epub 2016 Nov 14

106. Prior M, Stewart J, McEleny K, Dwyer AA, Quinton R. Fertility induction in hypogonadotropic hypogonadal men. Clin Endocrinol (Oxf). 2018;89:712-718. Epub 2018 Oct 9

107. Liu L, Banks SM, Barnes KM, Sherins RJ. Two-year comparison of testicular responses to pulsatile gonadotropin-releasing hormone and exogenous gonadotropins from the inception of therapy in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 1988;67:1140-1145.

108. Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H, Crowley WF Jr. Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2002;87:4128-4136.

109. Rees MC, Kuhl H, Engelstein M, Mattila L, Mäenpää J, Mustonen M; Study Groups. Endometrial safety and tolerability of triphasic sequential hormone replacement estradiol valerate/medroxyprogesterone acetate therapy regimen. Climacteric. 2004;7:23-32.

110. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, Lin AE, Mauras N, Quigley CA, Rubin K, Sandberg DE, Sas TCJ, Silberbach M, Söderström-Anttila V, Stochholm K, van Alfen-van derVelden JA, Woelfle J, Backeljauw PF; International Turner Syndrome Consensus Group. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. Eur J Endocrinol. 2017;177:1-70.

111. Matthews D, Bath L, Högler W, Mason A, Smyth A, Skae M. Hormone supplementation for pubertal induction in girls. Arch Dis Child. 2017;102:975-980. Epub 2017 Apr 26

REVIEW

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Current Diagnostic Approaches in the Genetic Diagnosis of Disorders of Sex Development

Deniz Özalp Kızılay, Samim Özen

Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, İzmir, Turkey

Abstract

Disorders of sex development (DSD) are a clinically and genetically highly heterogeneous group of congenital disorders. The most accurate and rapid diagnosis may be possible with a complementary multidisciplinary diagnostic approach, including comprehensive clinical, hormonal, and genetic investigations. Rapid and accurate diagnosis of DSD requires urgency in terms of gender selection and management of the case. Despite the genetic tests performed in current daily practice, the genetic cause is still not elucidated in a significant proportion of cases. Karyotype analysis can be used as a standard for sex chromosome identification. In addition, quantitative fluorescent-polymerase chain reaction or fluorescence *in situ* hybridization analysis can be used for faster and more cost-effective detection of the sex chromosome and *SRY* gene. Multiplex ligation-dependent probe amplification, single-gene sequence analysis, next-generation sequence analysis (NGSA), targeted NGSA, whole-exome sequencing, and whole-genome sequencing analyses can be performed according to preliminary diagnoses. Microarray analysis, including array comparative genomic hybridization and single nucleotide polymorphism array, should be performed in cases with syndromic findings and if no pathology is detected with other tests. In DSD cases, the use of optical genome mapping and techniques, which will probably be in daily practice in the near future, may be considered. In conclusion, the clinical and genetic diagnosis of DSD is difficult, and molecular genetic diagnosis is often not available. This has psychosocial and health implications for patients and their families. New genetic techniques, especially those targeting the whole genome, may provide a better understanding of DSD through the identification of little-known genetic causes. This review focuses on conventional genetic and next-generation genetic techniques used in the genetic diagnosis of DSD, as well as possible genetic diagnostic techniques and approaches that may be used in routine practice in the near future.

Keywords: Disorders of sex development, diagnostic approaches, genetic diagnosis

Introduction

Disorders of sex development (DSD) occur as a result of a disorder in one of the stages of sex development, especially in the first trimester due to incompatibility of chromosomes, gonads, or anatomical structure (1,2,3). DSDs are classified under three main headings: (i) sex chromosomal causes; (ii) 46, XY DSD; and (iii) 46, XX DSD (3,4). DSDs are observed in approximately 1 in 4,500-5,500 births and at least 50 different congenital urogenital differentiation anomalies have been defined to date (4,5). DSD is estimated to be more frequent in societies where consanguineous marriages are common. DSD is considered a medical, social, and forensic emergency in the neonatal and infancy periods because it involves many problems, especially in the first two years in affected cases, such as sexual identity development disorder, hormonal disorders, and psychosocial differences. Families ask questions just after birth about the clinical status and sex of the baby to the physicians who evaluate the newborn. Therefore, rapid, and accurate postnatal diagnosis of babies with DSD is very important (1,2,3,4,5).

DSDs are one of the most difficult groups of endocrine disorders to diagnose due to their genetic and clinical heterogeneity. Hence, a multidisciplinary approach is

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Address for Correspondence: Samim Özen MD, Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, İzmir, Turkey
E-mail: samim.ozen@ege.edu.tr, samimozen@gmail.com **ORCID:** orcid.org/0000-0001-7037-2713

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required from the very beginning of the evaluation of DSD patients, due to the highly complex molecular and hormonal etiological causes. Multiple genetic etiologies, ranging from missense single nucleotide variants (SNVs) to complete chromosome aneuploidies, have been demonstrated among the genetic causes of DSD. At least 75 genes have been associated with DSD in humans. With the genetic technologies developed in recent years, DSD cases can be diagnosed more quickly and accurately at lower costs (6,7,8).

Genetic Methods Used in the Diagnosis of DSD: Karyotype analysis and sex chromosome identification is the first step in the diagnostic approach to DSD. If sex chromosomal causes are identified by karyotype analysis, further analyses are not required. However, approximately 9% of cases have sex chromosome abnormalities, whereas higher-resolution molecular and molecular cytogenetic analyses are warranted in the majority of cases for genetic diagnosis (7,8,9). Cytogenetic and molecular diagnosis of biopsy tissues can rarely be performed when chromosomal anomalies, especially mosaicism, are considered.

Cytogenetic Investigation

Karyotype Analysis

Karyotype is the organization and classification of human chromosomes according to their size, shape, and banding patterns. In order to evaluate the organization and general morphology of human chromosomes by karyotype analysis, the cells to be used must be proliferated in culture. The most commonly used cells for karyotype analysis are white blood cells, particularly T lymphocytes. For cytogenetic analysis of these cells, "short-term culture", in which a peripheral blood sample is seeded in a tissue culture medium and then prepared to divide, is the most suitable method. After a few days, the dividing cells are arrested in mitosis using various chemicals that inhibit the mitotic cascade. In order to release the chromosomes, the cells are burst with a hypotonic solution added to the medium. Afterwards, they are made ready for staining by fixation and smearing (10). The work period is 1-2 weeks.

Karyotype analysis allows the detection of aneuploidy and/or mosaicism, and structural variants. In karyotype analysis, 20 metaphases are evaluated as standard in most laboratories. However, if necessary, especially if mosaic conditions are considered, up to 30-50 metaphases can be counted. In karyotyping with the standard G-banding, a total of approximately 400-550 bands can be visualized. This level of resolution allows the detection of deletions or duplications larger than approximately 5-10 Mb. The diagnostic efficiency in DSD is 15% and the majority of these are those with mosaic chromosome structure (6).

Molecular Cytogenetic Investigations

Fluorescence in situ Hybridization

The Fluorescence *in situ* hybridization (FISH) technique is a method that forms a bridge between molecular and cytogenetic examinations and is used for the detection of large and difficult to detect anomalies for molecular studies and small and undetectable anomalies for classical cytogenetic studies. It is used to investigate duplications larger than 500 kilobases (Kb) or deletions larger than 100 Kb. In this method, clones containing specific human DNA sequences, called "probes", are used to determine the presence or absence of the relevant region of the genome during metaphase or interphase. DNA probes can be prepared for an entire chromosome, only for a specific chromosome region or even only for gene-level targets. The probes are fluorescently labelled in different colors. They can be used to quickly determine the presence of abnormal chromosome numbers in clinical material or to detect chromosomal rearrangements. In FISH analysis, 100 or more metaphases allow 500 metaphases to be analyzed in an average-quality test. It provides more accurate information than karyotype analysis, especially in the detection and confirmation of structural and numerical chromosomal anomalies and mosaic conditions. Although FISH technology provides higher sensitivity and higher resolution than G-banding, it does not allow simultaneous analysis of all sex chromosomes and the whole genome (11).

Chromosomal Microarray

Copy number variations (CNVs) are one of the well-characterized causes of genetic diseases. Karyotype and microarray analyses are the gold standard methods for CNV detection. CNV is classically defined as changes greater than 1 Kb. The insertions, deletions, and duplications leading to CNVs are found throughout the genome in humans and affect approximately 12% of the human genome (12,13).

CNVs can occur at different frequencies in populations. When the frequency of CNV is more than 1%, it is called copy number polymorphism. In the general population, deletions are more common than duplications at rate of 2:1 (14). Deletions are more harmful than duplications because dosage-sensitive genes are unable to tolerate haploinsufficiency. CNVs containing more than one gene can have a wide spectrum of phenotypic effects due to the combined effect of different genes on a single phenotype or pleiotropic effects of a single gene on multiple phenotypes (6,7,13,14).

Chromosomal microarray is a molecular cytogenetic method that analyses CNVs in the DNA sequence across the whole genome. As a result of the combined use of molecular biological and robotic techniques, it is possible to perform gene expression analyses and genotyping of single nucleotide polymorphisms (SNPs) in cells with arrays obtained by adhering thousands of DNA fragments, each representing a specific gene on a glass matrix. The conventional cytogenetic methods frequently used in laboratories can detect chromosomal alterations of 5Mb (1 Mb = 1 million bases) or larger. With recent advances in chromosomal microarray analyses, the resolution limit of 5 Mb has decreased markedly (12).

Two frequently used types of microarray methods are array comparative genomic hybridization (aCGH) and single nucleotide polymorphism array (SNPa). CGH-based arrays measure the amount of genomic DNA. It compares the genomic DNA in a patient's sample with that in a normal control sample. Improvements in aCGH techniques have provided the opportunity to evaluate changes >1 Kb in size by comparing them with the reference genome (15). In SNPa, DNA probes derived from regions of a single base pair (BP) in the genome that show differences between individuals are used for CNV detection. It can determine the corresponding SNP genotype since each probe is present in SNPa (16). In addition, microarray method allows homozygosity mapping, detection of chimerism, uniparental disomies and inherited genetic identity, and diagnosis of polyploidy (15).

Array CGH and SNPa are methods that can detect submicroscopic genome imbalance and CNV as small as 10 Kb throughout the whole genome. In cases with normal karyotype analysis, especially syndromic DSD, aCGH and/or SNPa analysis should be performed in the absence of other known molecular causes. Microarray analysis offers a highly efficient and powerful whole genome screening opportunity instead of many diagnostic tests used in the identification of DSDs. Microarray method is also recommended as a first-line test, especially in syndromic cases with multiple congenital anomalies (6,7,17).

Molecular Genetic Investigations

Quantitative Fluorescent-polymerase Chain Reaction

Quantitative fluorescent-polymerase chain reaction (QF-PCR) is a method for the rapid identification of chromosomes 13, 18, and 21, each a major cause of numerical anoploidy in humans, as well as of the X and Y chromosomes and the *SRY* (Sex-Determining Region Y) gene by short tandem repeat analysis. The advantages of this method over other methods, including FISH, are that it is faster, more reliable, less costly, and requires less material. However, it should be kept in mind that especially mosaic conditions may not be detected by QF-PCR technique (18).

Multiplex Ligation-dependent Probe Amplification

Multiplex ligation-dependent probe amplification (MLPA) is a multiplex polymerase chain reaction-based technique that can detect dose changes for more than 50 regions in the genomic DNA or RNA sequence. It can distinguish even a SNV on the genome and is widely used in genetic laboratories as a simple, fast, low-cost, practical technique, unlike microarray and SNPa methods. MLPA can be used in the diagnosis of single gene diseases in which deletions/duplications are frequently seen as disease-causing changes (mutations), or in the diagnosis of diseases in which large deletions/duplications are suspected but appear normal after screening for SNVs. Array-CGH is another method that is suitable for detecting deletions and duplications. However, aCGH is an expensive method and MLPA should be preferred if deletion/duplication is being investigated in a known region or in a known gene(s). In addition to deletion/duplication analyses, it may provide preliminary information about the number of chromosomes and detect aneuploidies with probes arranged according to certain regions of the chromosomes. Limitations of this method are that it is not suitable for the detection of balanced translocations and point mutations, is vulnerable to contamination, and has lower resolution due to being targeted (usually single gene). MLPA is an analysis method designed to be limited to a single gene or gene groups. If more than one gene/gene groups are considered in the preliminary diagnosis, each gene needs to be analyzed separately, which leads to an increase in the analysis time and cost. Chromosomal microarray methods should be used when deletion/duplication of more than one gene/gene groups is considered (19,20).

Single Gene Sequence Analysis

DNA sequence analyses or sequencing methods are used to determine DNA primary (basic) structures. DNA sequence analysis has provided a lot of knowledge about genetic control mechanisms and gene structure. In order to understand the mechanisms related to the appearance and treatment processes of hereditary diseases, it is necessary to elucidate the gene regions that affect the disease under investigation. In this respect, DNA sequence analyses are the most important factor in determining the path to be followed at the beginning and during the course of the treatment process.

With conventional Sanger sequence analysis, short sequence reads (maximum 1000-1200 BP) can be performed and

each gene is analyzed individually in sequence according to the preliminary diagnosis. While this process provides highly reliable results, it is time consuming and costly. It should be kept in mind that gene dosage imbalances and large deletion and duplication mutations cannot be detected by Sanger sequence analysis. Therefore, Sanger sequence analysis is not a practical approach for routine use in cases where a high number of genes or large genes need to be analyzed. Large gene deletions and duplications constitute an important part of the molecular defect in DSD. For this reason, gene-specific MLPA, aCGH or SNPa analyses should be performed in cases where mutations cannot be detected by Sanger array analysis (6,7,8,9,21).

Next Generation Sequence Analysis

Next generation sequence analysis (NGSA) is based on enzymatically cutting DNA, creating a database with a large number of DNA fragments, and reproducing these DNA fragments. With parallel sequencing, millions of small DNA fragments are sequenced simultaneously, ensuring that each base in the genome is read more than once and variations can be detected more accurately. The main steps of the system can be summarized as follows: obtaining biological material to be studied; isolation of genomic DNA from the obtained biological materials; then selecting the target regions in the isolated DNA; creating a DNA database by cutting the DNA with an enzymatic reaction; reproducing the DNA fragments that make up the database; sequencing the DNA fragments; generation of raw data after sequencing; mapping against a reference sequence; identification and interpretation of possible changes; verification and segregation analysis by Sanger sequencing or NGS; identification of candidate pathogenic changes; and finally reporting of these data (22).

Targeted Next Generation Sequence Analysis Panels

With targeted NGSA panels, a large number of genes can be sequenced simultaneously in a shorter time for diseases with genetic heterogeneity in their etiology, such as DSD (23). The results can be more easily analyzed in a targeted NGSA than in clinical exome or genome analysis, as fewer variants will be identified. Thus, NGSA panels provide faster results compared to clinical exome and genome analyses. Due to genetically highly heterogeneous causes, targeted NGSA panels are very fast, highly successful and economical for molecular diagnosis in DSD cases (24).

Whole Exome Sequencing

Whole exome sequencing (WES) is a high-resolution technology with a relatively high diagnostic success rate that allows simultaneous analysis of the coding regions of more than 20,000 known genes in the human genome (25,26). WES is currently the most common technological approach used to analyze the protein-coding part of the human genome. Although WES covers only 1-1.5% of the human genome, even this small portion of the genome contains approximately 85% of the known mutations that cause disease. However, it can elucidate the genetic origin of diseases in only 25-40% of cases (27). This rate is higher than that obtained by more classical methods such as karyotype and chromosomal microarray (15-20%) (22). Although WES is a powerful diagnostic tool, it should be recognized that it is not the best diagnostic approach for all clinical indications and is the most important step in establishing the necessary relationships between clinical findings and the phenotype variants (28).

Whole Genome Sequencing

While single gene analyses, panel tests, and microarray analysis examine known variants in a previously identified gene, WES analysis only examines exon regions encoding functional proteins. In whole genome sequencing (WGS), all coded and non-coded regions of all genes in the human genome are sequenced. Thus, nucleotide changes that can cause genetically complex diseases can be fully analyzed. WGS enables the comprehensive identification of many variants simultaneously in a single gene analysis. Today, numerous clinical studies have revealed that non-coding sequence variants also play a critical role in the diagnosis of diseases. While 85% of information can be provided with WES, detailed information about the genome is provided by looking at non-coding variants, deletions, duplications, and CNVs encoded in "WGS" (27).

Clinical information and phenotypic characteristics of the patient are very important in the diagnostic success of WGS. If the clinical information is given in detail, it will be easier to find the relevant gene variant among thousands of genes. A very large variant database is also required for more successful phenotype-genotype matching and faster determination of the patient's diagnosis (29).

Diagnostic Approach for Disorders of Sex Development

Rapid and accurate diagnosis of DSD is urgent in terms of sex selection and management of the case in neonates. Incorrect and delayed diagnosis in the early period may cause serious and sometimes irreversible medical, anatomical, and psycho-social problems for the child and his/her family. The difficulty in diagnosis and the long duration of the diagnosis make the management of the case challenging for healthcare professionals and increase medical expenditures. For all these reasons, early, accurate, and rapid diagnosis is

very important in DSD cases. Currently, diagnosis of DSD cases takes a long time with classical hormonal and genetic analyses. One of the most important problems in the diagnosis of DSD is the high genetic heterogeneity (1,2,3,4). The first step in the diagnostic approach in DSD cases requires the determination of the patient's sex chromosome and the presence of the *SRY* gene. The gold standard test for sex chromosome determination is karyotype analysis. However, since the determination of sex chromosomes by karyotype analysis is quite time consuming, QF-PCR or FISH analysis can be used for rapid sex chromosome determination. FISH and QF-PCR analyses are also used to identify the presence of the *SRY* gene (3,4,6,7,9).

Following the determination of sex chromosomes and evaluation of the presence of the SRY gene, further molecular genetic analyses are required for the preliminary diagnosis based on clinical and hormonal findings. With the widely used Sanger sequence analysis, short sequence (maximum 1,000-1,200 BP) readings can be performed, and each gene is analyzed separately in sequence for preliminary diagnosis. This procedure is very time consuming and costly. In addition, large deletion and duplication mutations cannot be detected by Sanger sequence analysis. Large gene deletions and duplications constitute an important part of molecular defects in DSD. MLPA analysis specific to the relevant gene should be performed in patients who are thought to have a mutation in a specific gene according to the preliminary diagnosis but in whom mutation cannot be detected by Sanger sequence analysis (6,7,8,9).

Algorithms in current diagnostic flowcharts are often time-consuming, costly, and unsuitable to accurately and rapidly diagnose DSD, which is considered one of the endocrine urgencies.

NGSA is widely used in genetic research laboratories and clinical diagnostic centers. Whole genome, whole exome, or targeted gene analyses can be performed with NGSA. NGSA provides important advantages in the diagnosis of genetic heterogeneous diseases like DSD (30,31). Genes responsible for the etiology can be studied simultaneously with NGSA and thus, results can be obtained much more easily and in a shorter time compared to Sanger sequence analysis. It was shown by Özen et al. (24) that 45% of cases were diagnosed molecularly with a targeted NGSA gene panel in 46, XY DSD cases, the diagnostic time could be reduced to three days and the diagnostic cost was one-third of the conventional diagnostic approach. Despite all these advantages, the NGSA method has some limitations. Especially in cases where the reading depth is low, it may result in sequence errors and misalignment. It is not possible to detect large deletion or insertion mutations, triple nucleotide repeat regions, and some CNVs with NGSA due to short reads. This situation interferes with the holistic approach to the diagnosis of DSD with NGSA. Additional molecular analyses, such as MLPA or microarray analysis, are needed to demonstrate large deletions and duplications (30,31). Table 1 shows the characteristics of current and future genetic technologies used for the diagnosis of DSD (6).

The Approach to Be Followed in Genetic Diagnosis

Depending on the history, findings on physical examination, family history of DSD or reproductive problems, chromosomal sex, initial hormonal evaluation, presence of associated malformations, presence of functional testis or Müllerian structures, locally preferred or available genetic testing facilities, diagnostic pathways for the genetic diagnosis of DSD can be designed. The clinical and genetic diagnosis flowcharts for 46, XX DSD and 46, XY DSD are presented in Figures 1, 2.

In general, a preliminary diagnosis of DSD subgroup is first made by physical examination and hormonal evaluation, followed by sex chromosome identification. According to these preliminary diagnoses, targeted gene panels based on NGSA, WES and then WGS analyses can be performed. However, chromosomal microarray analysis should also be performed, especially in cases with syndromic findings and if no pathology is detected with other tests. In an infant with atypical external genital appearance, the presence of palpable gonads, the status of Müllerian structures, initial chromosome analyses, and hormonal evaluations may determine the genetic test to be selected according to the preliminary diagnosis. Currently, a multidisciplinary diagnostic approach is recommended from the beginning in genetically heterogeneous diseases like DSD.

Most genetic laboratories follow the American College of Medical Genetics and Genomics guidelines and use standard terminology ['pathogenic', 'likely pathogenic', 'variants of uncertain significance (VUS)', 'likely benign', and 'benign'] for the interpretation of variants obtained from sequence analysis of genes causing Mendelian inherited diseases.

Currently, the general recommendation is to report variants categorized as 'pathogenic', 'likely pathogenic', and 'VUS' in the gene(s) related to the patient phenotype (32). A multidisciplinary team consisting of pediatric endocrinology, genetics, and clinical biochemistry specialists is required in the evaluation of genetic results, especially "VUS" obtained from targeted gene panel and/or whole exome/genome sequencing and microarray technologies in cases related to DSD other than chromosome disorders. After the evaluation

Table 1. Characteristics and utilisation fields of genetic tests used in the diagnosis of DSD

Methodology	Operation time	Identifiable variants					Resolution	Diagnostic efficiency
		Aneuploidy and/or mosaicism	CNV	Encoded SNV	Uncoded SNV	Structural variant		
Clinically feasible methods								
Karyotype	1-4 weeks		X	X	X		> 5 Mb	15 % (mostly mosaics)
Interphase FISH (X, Y or SRY markers)	< 3 days		X	X	X	X	Inapplicable	Rapid gender decision
Microarray	2-3 weeks			X	X	X	< 50 Kb	15 %
Single-gene analysis or gene panel		X	X		X	X	SNV: 1 nucleotide indels: < 50 BP	Panel dependent
Whole exome sequencing		X	X		X	X	SNV: 1 nucleotide indels: < 50 BP	30-45 %
Whole genome sequencing		X	+ Requires verification			+ Requires verification	SNV: 1 nucleotide	At least 30-45 %
Preclinical, future methods								
Optical genome mapping				X	X		Structural variant: > 500 BP	?
"Long read" sequencing	?	X		X	X		Structural variant: 50 BP	?

BP: base pair, CNV: copy number variation, FISH: fluorescence *in situ* hybridization, Kb: kilobase, Mb: million bases, SNV: single nucleotide variant, SRY: sex-determining region Y, DSD: disorders of sex development

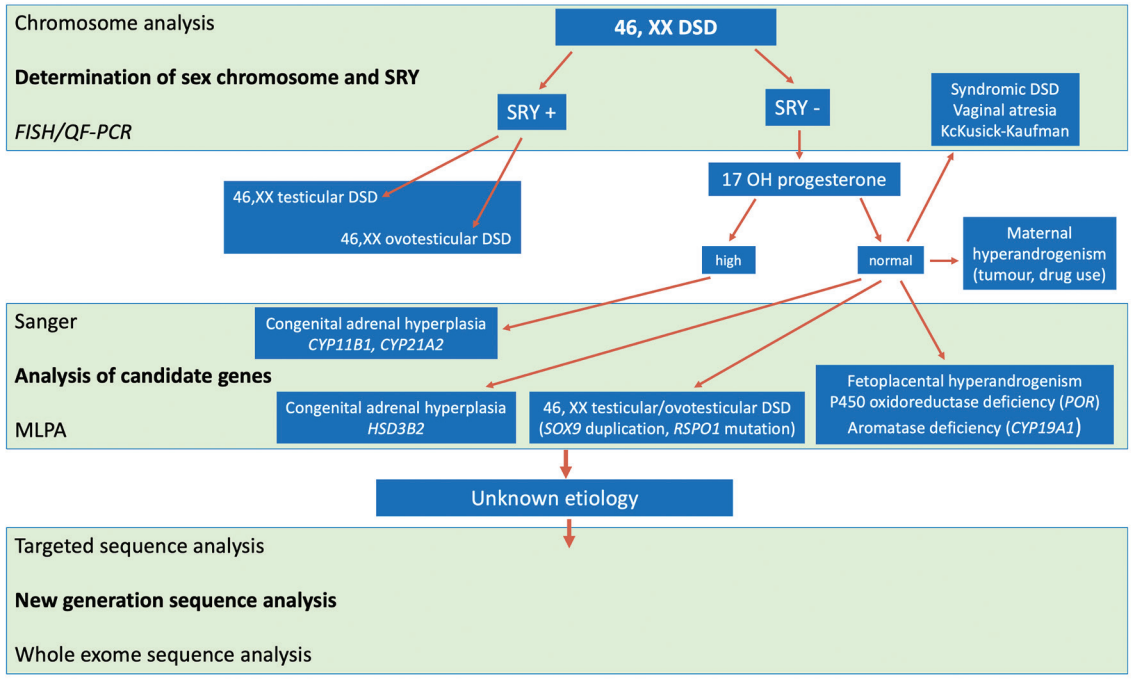

Figure 1. Genetic-based diagnostic approach pathway in 46 XX, DSD

CYP11B1: 11β-hydroxylase, CYP19A1: cytochrome P450 aromatase, CYP21A2: 21α-hydroxylase, DSD: disorders of sex development, FISH: fluorescence in situ hybridization, HSD3B2: 3-beta hydroxysteroid dehydrogenase 2, MLPA: multiplex ligation-dependent probe amplification, QF-PCR: quantitative fluorescent-polymerase chain reaction, RSPO1: R-spondin1, SOX9: SRY-box transcription factor 9

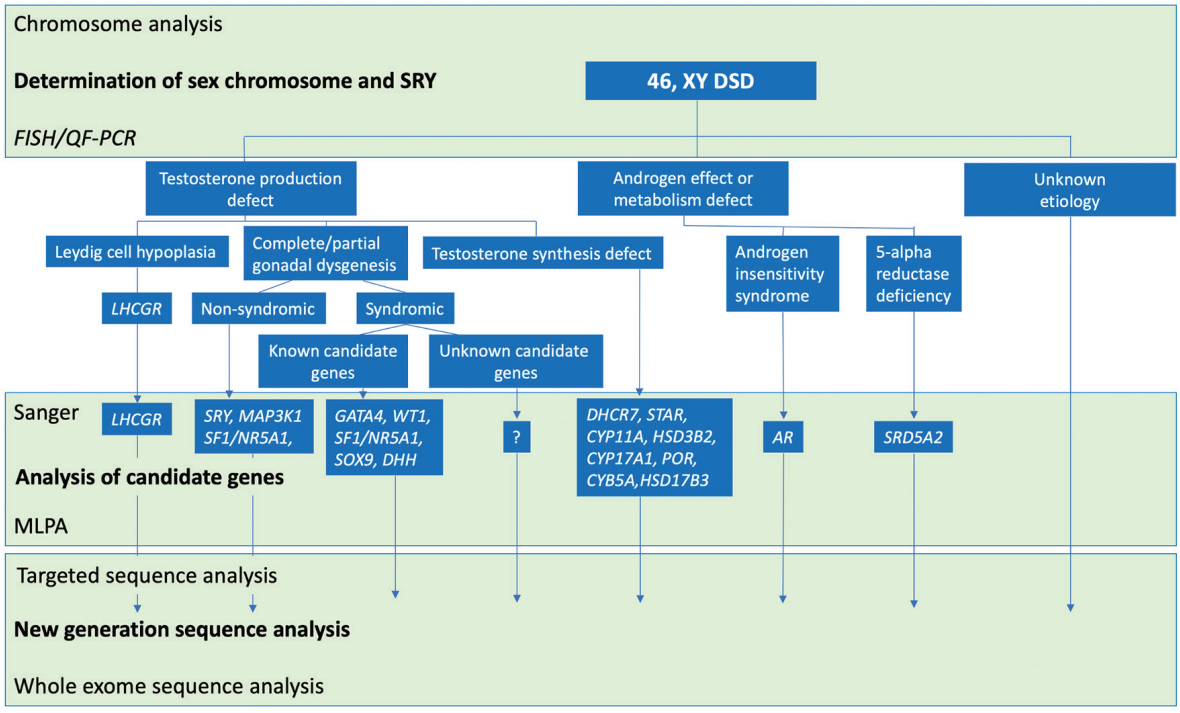

Figure 2. Genetic-based diagnostic approach pathway in 46 XY, DSD

AR: androgen receptor, CYB5A: cytochrome b5 type A, CYP11A1: P450 side-chain cleavage, CYP17A1: cytochrome P450, family 17, subfamily A, DHCR7: 7-dehydrocholesterol reductase, DHH: desert hedgehog signaling molecule, DSD: disorders of sex development, FISH: fluorescence in situ hybridization, GATA4: GATA binding protein 4, HSD17B3: 17β-hydroxysteroid dehydrogenase 3, HSD3B2: 3-beta hydroxysteroid dehydrogenase 2, LHCGR: luteinizing hormone/choriogonadotropin receptor, MAP3K1: mitogen-activated protein kinase 1, MLPA: multiplex ligation-dependent probe amplification POR: cytochrome P450 oxidoreductase, QF-PCR: quantitative fluorescent-polymerase chain reaction, SF1/NR5A1: steroidogenic factor 1, SOX9: SRY-box transcription factor 9, SRD5A2: steroid 5 alpha-reductase 2, STAR: steroidogenic acute regulatory protein, SRY: sex-determining region Y, WT1: WT1 transcription factor

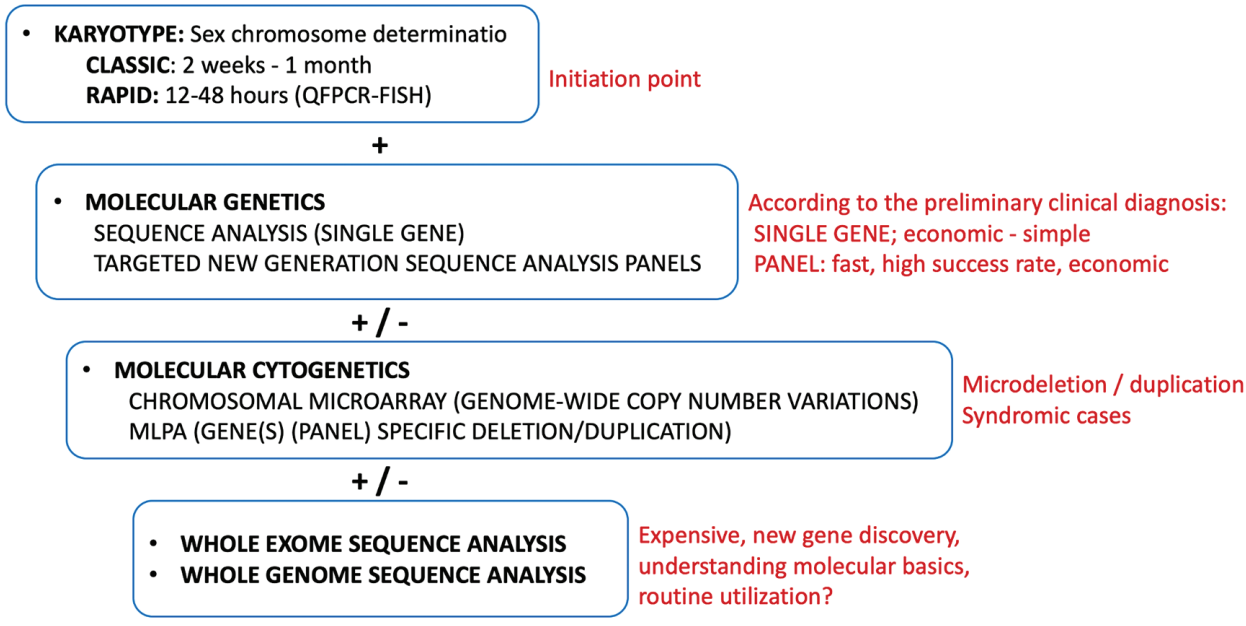

Figure 3. Characteristics of genetic tests used in the diagnosis of DSD

FISH: fluorescence in situ hybridization, QF-PCR: quantitative fluorescent-polymerase chain reaction, DSD: disorders of sex development

by the team, second-line endocrine tests and *in silico* and/or *in vitro* functional analyses should be planned to assess the association of any "VUS" with the disease.

Figure 3 shows the main characteristics of genetic tests used in the genetic diagnosis of DSD.

Conclusion

The first step in cases with suspected DSD is to determine the presence of sex chromosome and the *SRY* gene. Karyotype analysis can be used as a standard for sex chromosome detection. In addition, QF-PCR or FISH analysis can be used to detect the sex chromosome and *SRY* gene more rapidly and cost-effectively. However, it should be kept in mind that QF-PCR analysis cannot show mosaic conditions.

Microarray analysis (array-CGH and SNPa) showing high-resolution CNV throughout the WGS can be added to the first-line genetic tests, especially in DSDs with additional malformations, syndromic cases, and those in whom variants cannot be detected with other genetic tests.

MLPA can be used in the diagnosis of single gene diseases in which deletion/duplication is frequently seen as a disease-causing variation, or in the diagnosis of diseases in which a large deletion/duplication is suspected but was apparently negative after screening for SNVs. MLPA should be preferred if deletion/duplication is suspected in a gene(s) known to be associated with a DSD. Furthermore, in addition to deletion/duplication analyses, it may be possible to obtain preliminary information about the number of chromosomes and detect aneuploidies with probes arranged according to some regions of chromosomes.

In all newborns and small infants presenting with ambiguous external genitalia, potentially life-threatening acute adrenal insufficiency (e.g. forms of congenital adrenal hyperplasia such as 21-hydroxylase, 11ß-hydroxylase or 3ß-hydroxysteroid dehydrogenase deficiencies) should be urgently excluded. For this purpose, if a preliminary diagnosis can be established by first-line hormonal analyses and steroid profile measurement in urine and/or plasma together with history and clinical findings, Sanger sequence analysis can be promptly performed. In addition, targeted gene panels or WES analyses can be performed in these cases according to the clinical preliminary diagnosis and the facilities of the local genetic laboratory. In all other cases, clinical phenotyping, biochemical/hormonal analyses, and genetic tests should be planned simultaneously, through a multidisciplinary team. To confirm the cause of monogenic familial DSD, a simple and cost-effective gene and variant-specific Sanger sequence analysis can be performed. Moreover, a targeted gene panel of suspect genes or WES should preferably be used to analyze candidate genes in DSD cases with a highly heterogeneous genetic cause. WES is currently used for the investigation of new DSD genes, in cases where an oligogenic/polygenic basis of DSD is suspected, and for further research.

Despite the genetic tests performed in current daily practice, the genetic cause is still not elucidated in a significant proportion of cases. In these cases, the use of optical genome mapping and techniques, which will probably be in daily practice in the near future, may be considered.

Footnotes

Authorship Contributions

Design: Deniz Özalp Kızılay, Samim Özen, Literature Search: Deniz Özalp Kızılay, Samim Özen, Writing: Deniz Özalp Kızılay, Samim Özen.

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References

1. Ahmed SF, Bashamboo A, Lucas-Herald A, McElreavey K. Understanding the genetic aetiology in patients with XY DSD. Br Med Bull. 2013;106:67-89. Epub 2013 Mar 25
2. Mendonca BB, Domenice S, Arnhold IJ, Costa EM. 46,XY disorders of sex development (DSD). Clin Endocrinol (Oxf). 2009;70:173-187.
3. Hughes IA, Houk C, Ahmed SF, Lee PA; Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology Consensus Group. Consensus statement on management of intersex disorders. J Pediatr Urol. 2006;2:148-162. Epub 2006 May 23
4. Lee PA, Houk CP, Ahmed SF, Hughes IA; International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. Pediatrics. 2006;118:488-500.
5. Warne GL, Raza J. Disorders of sex development (DSDs), their presentation and management in different cultures. Rev Endocr Metab Disord. 2008;9:227-236. Epub 2008 Jul 17
6. Délot EC, Vilain E. Towards improved genetic diagnosis of human differences of sex development. Nat Rev Genet. 2021;22:588-602. Epub 2021 Jun 3

7. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM, Greenfield A, Bashamboo A, Hiort O, Wudy SA, McGowan R; The EU COST Action. Genetics in Endocrinology: Approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. Eur J Endocrinol. 2018;179:197-206.

8. Kremen J, Chan YM. Genetic evaluation of disorders of sex development: current practice and novel gene discovery. Curr Opin Endocrinol Diabetes Obes. 2019;26:54-59.

9. León NY, Reyes AP, Harley VR. A clinical algorithm to diagnose differences of sex development. Lancet Diabetes Endocrinol. 2019;7:560-574. Epub 2019 Feb 22

10. Montazerinezhad S, Emamjomeh A, Hajieghrari B. Chromosomal abnormality, laboratory techniques, tools and databases in molecular Cytogenetics. Mol Biol Rep. 2020;47:9055-9073. Epub 2020 Oct 26

11. Fantes JA, Boland E, Ramsay J, Donnai D, Splitt M, Goodship JA, Stewart H, Whiteford M, Gautier P, Harewood L, Holloway S, Sharkey F, Maher E, van Heyningen V, Clayton-Smith J, Fitzpatrick DR, Black GC. FISH mapping of de novo apparently balanced chromosome rearrangements identifies characteristics associated with phenotypic abnormality. Am J Hum Genet. 2008;82:916-926. Epub 2008 Mar 27. Erratum in: Am J Hum Genet. 2008;82:1019.

12. Gross AM, Ajay SS, Rajan V, Brown C, Bluske K, Burns NJ, Chawla A, Coffey AJ, Malhotra A, Scocchia A, Thorpe E, Dzidic N, Hovanes K, Sahoo T, Dolzhenko E, Lajoie B, Khouzam A, Chowdhury S, Belmont J, Roller E, Ivakhno S, Tanner S, McEachern J, Hambuch T, Eberle M, Hagelstrom RT, Bentley DR, Perry DL, Taft RJ. Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease. Genet Med. 2019;21:1121-1130. Epub 2018 Oct 8

13. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet. 2006;7:85-97.

14. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. Nature. 2006;444:444-454.

15. Keren B. The advantages of SNP arrays over CGH arrays. Mol Cytogenet. 2014;7(Suppl 1):31.

16. Levy B, Burnside RD. Are all chromosome microarrays the same? What clinicians need to know. Prenat Diagn. 2019;39:157-164. Epub 2019 Feb 10

17. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010;86:749-764.

18. Plaseski T, Noveski P, Trivodalieva S, Efremov GD, Plaseska-Karanfilska D. Quantitative fluorescent-PCR detection of sex chromosome aneuploidies and AZF deletions/duplications. Genet Test. 2008;12:595-605.

19. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30:e57.

20. Hawkins SFC, Guest PC. Multiplex Analyses Using Real-Time Quantitative PCR. Methods Mol Biol. 2017;1546:125-133.

21. Beck TF, Mullikin JC; NISC Comparative Sequencing Program; Biesecker LG. Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. Clin Chem. 2016;62:647-654. Epub 2016 Feb 4

22. Buermans HP, den Dunnen JT. Next generation sequencing technology: Advances and applications. Biochim Biophys Acta. 2014;1842:1932-1941. Epub 2014 Jul 1

23. Slatko BE, Gardner AF, Ausubel FM. Overview of Next-Generation Sequencing Technologies. Curr Protoc Mol Biol. 2018;122:e59.

24. Özen S, Onay H, Atik T, Solmaz AE, Özkınay F, Gökşen D, Darcan Ş. Rapid Molecular Genetic Diagnosis with Next-Generation Sequencing in 46,XY Disorders of Sex Development Cases: Efficiency and Cost Assessment. Horm Res Paediatr. 2017;87:81-87. Epub 2016 Nov 30

25. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A, Landsverk M, Craigen WJ, Bekheirnia MR, Stray-Pedersen A, Liu P, Wen S, Alcaraz W, Cui H, Walkiewicz M, Reid J, Bainbridge M, Patel A, Boerwinkle E, Beaudet AL, Lupski JR, Plon SE, Gibbs RA, Eng CM. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014;312:1870-1879.

26. Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, Das K, Toy T, Harry B, Yourshaw M, Fox M, Fogel BL, Martinez-Agosto JA, Wong DA, Chang VY, Shieh PB, Palmer CG, Dipple KM, Grody WW, Vilain E, Nelson SF. Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA. 2014;312:1880-1887.

27. Sawyer SL, Hartley T, Dyment DA, Beaulieu CL, Schwartzentruber J, Smith A, Bedford HM, Bernard G, Bernier FP, Brais B, Bulman DE, Warman Chardon J, Chitayat D, Deladoëy J, Fernandez BA, Frosk P, Geraghty MT, Gerull B, Gibson W, Gow RM, Graham GE, Green JS, Heon E, Horvath G, Innes AM, Jabado N, Kim RH, Koenekoop RK, Khan A, Lehmann OJ, Mendoza-Londono R, Michaud JL, Nikkel SM, Penney LS, Polychronakos C, Richer J, Rouleau GA, Samuels ME, Siu VM, Suchowersky O, Tarnopolsky MA, Yoon G, Zahir FR; FORGE Canada Consortium; Care4Rare Canada Consortium; Majewski J, Boycott KM. Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: time to address gaps in care. Clin Genet. 2016;89:275-284. Epub 2015 Sep 22

28. Tetreault M, Bareke E, Nadaf J, Alirezaie N, Majewski J. Whole-exome sequencing as a diagnostic tool: current challenges and future opportunities. Expert Rev Mol Diagn. 2015;15:749-760. Epub 2015 May 9

29. Nisar H, Wajid B, Shahid S, Anwar F, Wajid I, Khatoon A, Sattar MU, Sadaf S. Whole-genome sequencing as a first-tier diagnostic framework for rare genetic diseases. Exp Biol Med (Maywood). 2021;246:2610-2617. Epub 2021 Sep 15

30. Hughes LA, McKay-Bounford K, Webb EA, Dasani P, Clokie S, Chandran H, McCarthy L, Mohamed Z, Kirk JMW, Krone NP, Allen S, Cole TRP. Next generation sequencing (NGS) to improve the diagnosis and management of patients with disorders of sex development (DSD). Endocr Connect. 2019;8:100-110.

31. Dong Y, Yi Y, Yao H, Yang Z, Hu H, Liu J, Gao C, Zhang M, Zhou L, Asan, Yi X, Liang Z. Targeted next-generation sequencing identification of

mutations in patients with disorders of sex development. BMC Med Genet. 2016;17:23.

32. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424. Epub 2015 Mar 5

ORIGINAL ARTICLE

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Predictors and Trends of Diabetic Ketoacidosis at Diagnosis of Type 1 Diabetes Mellitus in Malaysian Children

Meenal Mavinkurve[1], Nurul Hanis Ramzi[2], Muhammad Yazid Bin Jalaludin[3], Nurshadia Samingan[3], Azriyanti Anuar Zaini[3]

[1]IMU University, School of Medicine, Department of Paediatrics, Seremban, Malaysia
[2]IMU University, Institute for Research, Development and Innovation, Kuala Lumpur, Malaysia
[3]Universiti Malaya Faculty of Medicine, Department of Paediatrics, Kuala Lumpur, Malaysia

What is already known on this topic?

The average diabetic ketoacidosis (DKA) rate in Malaysian children with type 1 diabetes mellitus ranged between 54-75% between 2000-2010.

What this study adds?

The DKA rate has remained persistently high since the year 2000 and severe DKA comprised the largest proportion. 96% of children under five year presented in DKA. Predictive factors for DKA were age ≥5 years and misdiagnosis. There were no significant trends in the rates of children <5 years presenting in DKA nor the rates of severe DKA.

Abstract

Objective: Previous reports indicate that diabetic ketoacidosis (DKA) rates in Malaysian children with type 1 diabetes mellitus (T1DM) range between 54-75%, which is higher than most European nations. Knowledge of trends and predictors of DKA can be helpful to inform measures to lower the rates of DKA. However, this data is lacking in Malaysian children. Hence, the aim of this study was to determine the predictors and trends of DKA in Malaysian children at the initial diagnosis of T1DM.

Methods: This cross-sectional study examined demographic, clinical and biochemical data of all newly diagnosed Malaysian children aged 0-18 years with T1DM over 11 years from a single centre. Regression analyses were used to determine predictors and trends.

Results: The overall DKA rate was 73.2%, 54.9% of the DKA cases were severe. Age ≥5 years [odds ratio (OR): 12.29, 95% confidence interval (CI): 1.58, 95.58, p=0.017] and misdiagnosis (OR: 3.73, 95% CI: 1.36, 10.24 p=0.01) were significant predictors of a DKA presentation. No significant trends in the annual rates of DKA, severe DKA nor children <5 years presenting with DKA were found during study period.

Conclusion: DKA rates at initial diagnosis of T1DM in Malaysian children are high and severe DKA accounts for a notable proportion of these. Though misdiagnosis and age ≥5 years are predictors of DKA, misdiagnosis can be reduced through better awareness and education. The lack of downward trends in DKA and severe DKA highlights the urgency to develop measures to curb its rates.

Keywords: Diabetic ketoacidosis, childhood, Malaysia, type 1 diabetes, trend

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Address for Correspondence: Azriyanti Anuar Zaini MD, Universiti Malaya Faculty of Medicine, Department of Paediatrics, Kuala Lumpur, Malaysia
E-mail: azriyanti@ummc.edu.my **ORCID:** orcid.org/0000-0001-5659-765X

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune condition, which peaks in children between 10-14 years of age (1). There is a wide variation in the incidence of T1DM worldwide, with higher incidence rates reported in Northern Europe as compared to Western Africa and South America (2,3). Within Asia, childhood T1DM rates have been rising in several regions, including Thailand, Hong Kong and Indonesia (4,5,6,7). In Malaysia, T1DM is the most common type of childhood diabetes, accounting for >69% of all diabetes mellitus cases. The International Diabetes Federation World Diabetes Atlas reported that in Malaysia in 2021, the estimated number of new cases of T1DM in the 0-19 age group was 100 and that the total number of cases was 1000 (5). A prior study by Hong et al. (8,9), showed that diabetic ketoacidosis (DKA) at diagnosis occurs in 65% of paediatric T1DM cases in Malaysia; however, analysis of the risk factors for DKA nor its trends have been conducted.

Paediatric DKA (pDKA) is a severe and potentially fatal presentation of T1DM that is characterised by hyperglycaemia, dehydration, ketosis and acidosis (10). Early recognition and management of pDKA are essential for reducing mortality, morbidity and financial burden. The incidence of pDKA at initial diagnosis of T1DM varies between countries, with lower rates reported in Northern Europe and higher rates in other regions, such as the United Arab Emirates, Saudi Arabia, Kuwait, Malaysia and Indonesia (11,12,13,14). This variation may be explained by several contributing factors, including age, socioeconomic status, delayed diagnosis or misdiagnosis, poor public awareness, educational background of the parents and the background frequency of paediatric T1DM in the population (15,16,17).

Trend analysis of pDKA at initial diagnosis of T1DM in children and determination of its associated risk factors has been conducted in several countries. Data from such studies are important because these may be informative in terms of developing interventions to reduce the incidence of pDKA. A recent epidemiological study from Thailand has reported that there has been a reduction in pDKA over the 20-year study period (4). New Zealand and Indonesia, on the other hand, have reported a sustained high incidence of pDKA at initial diagnosis or a rise in the incidence over the respective study periods (6,18). In Malaysia, Hong et al. (9), reported that the rate of pDKA at initial diagnosis of T1DM in 490 children from multiple centres between 2000-2010 varied between 54-75% of new cases. Though they reported that pDKA rates were mostly high throughout the study period, there was no evaluation of severe DKA rates nor a determination of the predictors of pDKA. Furthermore, data on trends in pDKA in Malaysian children with T1DM is limited since the study by Hong et al. (9) Nonetheless, their data highlighted the high rates of pDKA which is undoubtedly associated with significant morbidity and financial costs (19,20). In view of the burden associated with DKA, it is important that collective efforts are made to reduce the incidence of pDKA. Hence the objective of this study was to determine the predictors of pDKA at initial diagnosis of T1DM and to describe its trends in Malaysian children over an 11-year period.

Experimental Subjects

All newly diagnosed cases of paediatric T1DM in Malaysian children who were managed at Universiti Malaya Medical Centre (UMMC) between January 1st 2010 to December 31st 2020 were included. A diagnosis of T1DM and/or DKA was made in accordance with the International Society for Pediatric and Adolescent Diabetes (ISPAD) guideline for the year in which the diagnosis was made. Body mass index status was categorised using the World Health Organization Z-score cut-offs (21). Non-T1DM diabetes, non-Malaysians and subjects with incomplete data concerning DKA at diagnosis were excluded from the analysis.

Methods

A cross-sectional study was conducted using retrospective data that was extracted from the hospital electronic medical record system and letters from the referring physicians. Details on age, gender, ethnicity, DKA, misdiagnosis, anthropometry, intensive care admission and inpatient stay were obtained. DKA was defined as children presenting with hyperglycaemia (blood glucose >11 mmol/L) acidosis with a venous pH <7.3 or bicarbonate <15 mmol/L and ketonaemia or ketonuria. Mild cases were those with either venous pH <7.3 or bicarbonate <15 mmol/L, moderate cases were those with either a venous pH <7.2 or bicarbonate <10 mmol/L and severe were those with a venous pH <7.1 or bicarbonate <5 mmol/L. These definitions were in accordance with the ISPAD relevant to the year the diagnosis was made in. Misdiagnosis was defined as defined as any case that was given a diagnosis *other than diabetes* mellitus by a physician at UMMC. UMMC has an electronic medical record system with a proforma for in-patient admission clerking, into which details on the presenting history are entered on admission. All new diagnoses of paediatric T1DM are always admitted as inpatients, irrespective of whether they present in DKA or not, and all are reviewed by the Paediatric Endocrinology team.

This study was approved by the UMMC Institutional Ethics Board MREC ID no: 2019325-7251, date: 29.04.2019.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows, version 28.0 (SPSS Corp., Chicago, IL, USA) was used for statistical analysis. Demographic, clinical and biochemical data were analysed using descriptive statistics; mean ± standrad deviation for continuous variables and frequencies or percentages for categorical variables. Comparison of DKA and non-DKA groups were conducted using independent Student's t-test and Pearson's chi-squared test (χ^2) for continuous and categorical variables respectively. A logistic regression model was used to determine the predictors of DKA at initial diagnosis of T1DM. Gender and ethnicity were adjusted for as potential confounders. The odds ratios (OR) along with the respective 95% confidence intervals (CIs) were reported. The trend in DKA incidence rates over the 11-year period was analysed using Poisson regression. A two-sided 5% significance level was used for all statistical inferences.

Results

Demographic, Clinical and Biochemical Characteristics of the Overall Cohort

A total of 127 children aged 0-18 years with T1DM were identified during the 11-year study period. Males constituted 46.5% (n = 59) and the mean age of the cohort was 8.06 ± 3.78 years. Children ≥5 years comprised 78.7% (n = 100) of the whole cohort. The predominant ethnic group was Malay, 39.4 % (n = 50). The overall rate of DKA at presentation was 73.2% (n = 93) of which more than half were severe DKA (Table 1).

Diabetic Ketoacidosis vs. Non-diabetic Ketoacidosis Groups

The DKA group was significantly younger at diagnosis (7.64 ± 4.03 vs. 9.19 ± 2.77 years, p = 0.03) with 72.6% (n = 69) of pDKA group being represented by the ≥5 years age category (p = 0.003). Notably, 26 of 27 (96%) new diagnoses of T1DM presenting < 5 years of age had DKA. Misdiagnosis rates were significantly higher in the DKA group (43% vs. 17.6%, p = 0.004), as were pediatric intensive care units (PICU) admission rates (57.1% vs. 12%, p < 0.001) and the length of hospital stay (7.72 vs. 5.90, p = 0.01) (Table 2). Comparison of the three categories of severity of DKA showed that the severe DKA group had a significantly higher rate of admission to PICU (p = 0.001) (Table 3).

Predictors of Paediatric Diabetic Ketoacidosis: Logistic Regression Analysis

Binary logistic regression modelling, using DKA and non-DKA groups as the dependent variables, showed that age was a significant predictor of pDKA with ≥5 years age group (OR: 12.29, 95% CI: 1.58, 95.85, p = 0.017) was approximately 12 times more likely to have DKA. Similarly, misdiagnosis was determined to be a significant predictor of DKA (OR: 3.73, 95% CI: 1.36, 10.24, p = 0.01).

Trends in Diabetic Ketoacidosis Over the Decade

The annual rate of DKA varied from between 20% and peaking at 85% in 2015 (Figure 1). The rates of severe DKA fluctuated between 28.6% to 100% over the 11-year study period. In terms of age group, the percentage of children < 5 years of age who presented in DKA at the initial diagnosis of T1DM, varied from 0-46%. The lowest rates were in 2011-2012 and the highest in 2017.

Poisson regression analysis demonstrated that there were no significant increasing nor decreasing trends in the annual

Table 1. Demographic and clinical characteristics at diagnosis of T1DM

Age at diagnosis (years)	
Mean (± SD)	8.06 (± 3.78)
Age group, n (%)	
< 5 years	27 (21.3)
≥5 years	100 (78.7)
Gender, n (%)	
Male	59 (46.5)
Female	68 (53.5)
Ethnicity, n (%)	
Malay	50 (39.4)
Chinese	42 (33.1)
Indian	35 (27.6)
BMI status, n (%)[¥]	
Underweight	19 (20.7)
Normal weight	61 (66.3)
Overweight or obese	12 (13.0)
Blood glucose level (mmol/L)[#]	
Mean (± SD)	27.13 (9.03)
HbA1c (IFCC) at diagnosis (mmol/mol)[#]	
Mean (± SD)	115 (5)
Presence of DKA, n (%)	
DKA	93 (73.2)
Non-DKA	34 (26.8)
Severity of DKA, n (%)[*]	
Mild	19 (23.2)
Moderate	18 (22.0)
Severe	45 (54.9)

Data is presented as mean (± SD) for continuous variables (age, and biochemical parameters) and as a frequency and percentage for categorical variable. All percentages were calculated accounting for missing data.
[¥]Data was analysed for n = 92.
[#]Data was analysed for n = 107.
[*]Data was analysed for n = 82.
[$]Data was analysed for n = 50.
SD: standard deviation, DKA: diabetic ketoacidosis, T1DM: type 1 diabetes mellitus, IFCC: International Federation of Clinical Chemistry and Laboratory Medicine

Table 2. A comparison of DKA vs. non-DKA cases

	DKA (n = 93)	Non-DKA (n = 34)	p value
Age (year)			
Mean age (± SD)	7.65 (4.03)	9.19 (2.77)	**0.03**
< 5 years	26 (27.4%)	1 (2.9%)	**0.003**
≥5 years	67 (72.6%)	33 (97.1%)	
Gender			
Male	43 (46.2%)	16 (47.1%)	0.93
Female	50 (53.8%)	18 (52.9%)	
Ethnicity			
Malay	41 (44.1%)	9 (26.5%)	0.16
Chinese	27 (29.0%)	15 (44.1%)	
Indian	25 (26.9%)	10 (29.4%)	
BMI status[¥]			
Underweight	18 (26.5%)	1 (4.2%)	0.07
Normal weight	42 (61.8%)	19 (79.2%)	
Overweight or obese	8 (11.8%)	4 (16.7%)	
HCP contact prior to diagnosis[*]			
Less than 2	55 (70.5%)	23 (85.2%)	0.13
2 or more	23 (29.5%)	4 (14.8%)	
Misdiagnoses, n (%)[**]			
Misdiagnosis	40 (43.0%)	6 (17.6%)	**0.004**
Biochemical parameters			
Mean pH (± SD)	7.07 (0.16)	7.33 (0.15)	**< 0.001**
Mean bicarbonate (mmol/l) (± SD)	8.48 (4.77)	18.64 (5.74)	**< 0.001**
Mean glucose (mmol/l) (± SD)	28.47 (8.56)	23.26 (9.39)	**0.01**
Mean HbA1c, IFCC (mmol/mol) (± SD)	115 (7.0)	115 (1.0)	**0.89**
Current HbA1c, IFCC (mmol/mol)	80 (3)	77 (5.0)	**0.68**
PICU admission, n (%)[#]			
Yes	40 (57.1)	3 (12.0)	**< 0.001**
No	26 (37.1)	22 (88.0)	
Length of hospital stay, days (± SD)	7.72 (2.74)	5.90 (2.22)	**0.01**

Data is presented as mean (± SD) for continuous variables (age, biochemical parameters, and length of hospital stay) and as a frequency and percentage for categorical variable. Comparisons between T1DM participants with DKA versus those who did not have DKA used independent t-test for continuous variables and χ^2 test between categorical variables. Significant findings appear in bold.
[¥]Data was analysed for n = 92.
[*]Data was analysed for n = 105.
[**]Data was analysed for n = 46.
[#]Data was analysed for n = 91.
SD: standard deviation, DKA: diabetic ketoacidosis, T1DM: type 1 diabetes mellitus, IFCC: International Federation of Clinical Chemistry and Laboratory Medicine, PICU: pediatric intensive care units, BMI: body mass index

rates of DKA (p = 0.09), rates of severe DKA (p = 0.64) nor the rates of younger age children (< 5 years) presenting in DKA (p = 0.70) at initial diagnosis, over the 11 years.

Discussion

This single centre study over 11 years showed an overall pDKA rate at initial diagnosis of T1DM in Malaysian children of 73.2%. A disproportionately large percentage of the cases were severe DKA (54.9%). Children presenting in DKA were significantly younger than those presenting with new T1DM but without DKA. The DKA group was more likely to be misdiagnosed and require PICU admission with a longer length of inpatient stay. In particular, PICU admission rates were significantly higher in severe DKA cases. Logistic regression analysis demonstrated that children ≥5 years and misdiagnosis were the two main predictors of pDKA in this cohort. No significant increasing nor decreasing trends were demonstrated in the incidence of pDKA, rates of severe DKA, nor the rates of young children (< 5 years) presenting in DKA at diagnosis over the 11-year study period.

Rates of Diabetic Ketoacidosis

This is the second study to investigate the annual incidence of pDKA in Malaysian children at initial diagnosis of T1DM. A previous multicentre study by Hong et al. (9), reported an overall pDKA rate of 64.7%. Over their 10-year study period, the pDKA rate fluctuated between 54.5% and 75%. The mean age of their cohort presenting in DKA at diagnosis was 7.2 years and 70.4% of their < 5-year-old cohort presented in DKA. Gender and ethnicity were not different between the DKA and non-DKA groups. In the context of the study by Hong et al. (9), our study highlights that DKA rates in Malaysian children have remained high since 2010 and have failed to diminish over the last 20 years. Though rates of DKA in the current 11-year study also fluctuated, it never fell below 20%. Furthermore, a finding that was not previously reported is that this high burden of DKA is characterized by a high rate of severe DKA cases. Interestingly, over the last 20 years, the average age of pDKA has remained stable, 7.2 year in the earlier study and 7.65 year in the current study. However, though the mean age of children presenting with DKA is represented by the "school-going" age group, it is important to note that the frequency of DKA was higher in children < 5 years; 70.4% in the study of Hong et al. (9) compared with 96.3% in the current study.

Malaysian rates of pDKA are significantly higher than several Northern European countries but comparable to those reported within the Association of Southeast Asian Nations (ASEAN) region (22,23,24,25,26). We hypothesize that the sustained high rates of pDKA, are related to several factors.

Table 3. Demographics at presentation of children with DKA at diagnosis of T1DM according to DKA severity

	Mild DKA (n = 19)	Moderate DKA (n = 18)	Severe DKA (n = 45)	p value
Age at diagnosis (years)	9.04 (4.01)	7.42 (3.72)	7.32 (4.20)	0.29
Gender (males) %	47.4	44.4	46.7	0.99
Ethnicity %				
Malay	47.4	38.9	46.7	0.69
Chinese	26.3	44.4	26.7	
Indian	26.3	16.7	26.7	
BMI SDS¥	-1.37 (1.77)	-1.19 (1.62)	-0.81 (1.83)	0.61
Biochemical parameters, mean (± SD)				
pH	7.26 (0.05)	7.16 (0.03)	6.95 (0.10)	**< 0.001**
Bicarbonate (mmol/l)	13.18 (4.22)	10.22 (2.97)	5.39 (1.93)	**< 0.001**
Glucose (mmol/l)	26.31 (7.63)	28.21 (9.25)	29.30 (8.78)	0.47
HbA1c, IFCC (mmol/mol)	129 (9.0)	111 (0)	113 (5.0)	0.10
PICU admission, n (%)¥	2 (15.4)	3 (25.0)	32 (88.9)	**< 0.001**
Length of hospital stay, days, mean (± SD)	6.46 (3.37)	7.47 (2.23)	8.31 (2.65)	0.11

Data is presented as mean (± SD) for continuous variables (age, biochemical parameters, and length of hospital stay) and as a frequency and percentage for categorical variable. Comparisons between DKA severity groups used independent t-test for continuous variables and χ^2 test between categorical variables. Significant findings appear in bold.

¥Data was analysed for n = 61.

SDS: standard deviation (SD) score, DKA: diabetic ketoacidosis, BMI: body mass index, T1DM: type 1 diabetes mellitus, IFCC: International Federation of Clinical Chemistry and Laboratory Medicine, PICU: pediatric intensive care units

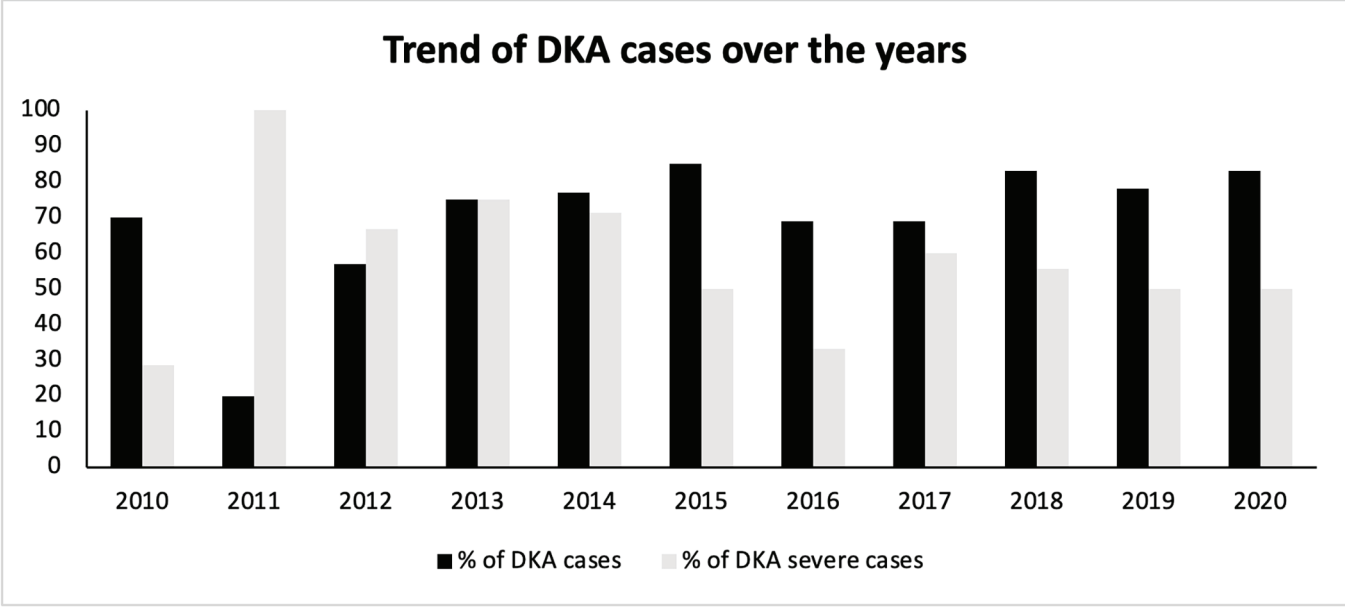

Figure 1. Percentage distribution of total and severe DKA cases at initial diagnosis of T1DM from 2010 to 2020 (n = 127)

DKA: diabetic ketoacidosis, T1DM: type 1 diabetes mellitus

These may include a lower background prevalence rate of T1DM in Malaysian children and potentially a reduced awareness that T1DM is a disease of childhood amongst the general public as well as differences in healthcare system structures. However, though these factors have been shown to correlate with high DKA rates in other countries, they have yet to be studied in the Malaysian context as potential risk factors for DKA and would require multicentre prospective studies (14,15).

Predictors of Diabetic Ketoacidosis at Initial Diagnosis of Type 1 Diabetes Mellitus

This study showed that the predictors of DKA at initial diagnosis of T1DM in Malaysian children were age ≥5 years and misdiagnosis. The study by Hong et al. (9), did show that school-aged children comprised the largest proportion of children presenting in DKA. This study expands on these earlier findings by showing that age ≥5 years is indeed a

predictor of DKA at initial diagnosis of T1DM. This finding is unexpected and contrary to other studies, which report that age <5 years is a risk factor for DKA, for the reasons that younger children present with less discernible symptoms and they lack the eloquence to explain their symptoms which may lead to diagnostic delays and errors (15,16). It is possible that our finding is influenced by the fact that a large proportion of our cohort (78.7%) were of school-going age, which is not dissimilar from the cohort in the study by Hong et al. (9). Nonetheless, both studies do demonstrate that the incidence of DKA in the <5-year-old age group was comparably higher than in older children, and so should still remain a cause for concern.

Misdiagnosis was another predictor of pDKA, increasing the risk of presenting in DKA by 3.5-fold which is in line with prior studies which have reported that misdiagnosis is a risk factor for DKA (15,16). However, unlike age, misdiagnosis is a modifiable factor for pDKA, suggesting that future efforts should focus on improving the diagnostic accuracy of pDKA by doctors through continuing professional development and implementing the recent ISPAD 2022 DKA guideline, which recommends that all children who present with breathlessness or vomiting and abdominal pain without diarrhoea should have a finger prick glucose performed as these signs and syptoms may herald DKA (27).

Trends

This study did not demonstrate any significant increasing nor decreasing trends in the annual incidence of DKA at diagnosis. However, it is important to note that the annual rates of DKA never fell below 20%. New Zealand and Austria have also reported that the incidence of pDKA has remained stable over a period of time (13,18) and the SEARCH study in the US, showed that the rates of pDKA with T1DM between 2002-2010 were sustainedly high without any reprieve (28). Within the ASEAN region, Thailand has shown that the rates of pDKA have been reducing (29).

The trends in the annual rates of severe DKA in this study were also not significant, but never fell below 30%. These findings are not unlike what was reported by a paediatrics DKA study from New Zealand which showed high rates of severe DKA that fluctuated between 10-40% over the 15-year study period (18). On the other hand, a study from China reported that their rates of severe DKA had increased in the younger age groups (29).

The proportion of children <5 years presenting in DKA at their initial diagnosis of T1DM did not demonstrate any significant trends but fluctuated between 0-46% over the 11 years. There were some years where there were no DKA presentations in <5 year old children, for the reason that only children ≥5 years were diagnosed with T1DM in those years. This is contrary to epidemiological data from New Zealand, Italy and Finland which show that rates of DKA in children <5 year are increasing over time (12,18,23,30). This result may be explained by the fact that Malaysian children with T1DM presenting in DKA are predominantly represented by the ≥5 year age group.

The wide variation in trends of pDKA between nations may be related to a multitude of factors, such as differences in the local prevalence of T1DM and public awareness of childhood T1DM, amongst others. A study conducted in New Zealand has demonstrated that in a group of 263 children the factors which contributed to an increased risk of DKA were reduced family awareness, prolonged delay in laboratory testing and a low level of health care professional suspicion for T1DM (31). Thus, preventing a DKA presentation at initial diagnosis of T1DM requires several key components which include: a) early recognition of symptoms by the parents and child; b) clinical suspicion of diabetes mellitus by the healthcare professional; and c) easy access to a medical professional with the appropriate point of care testing to diagnose diabetes mellitus. These three elements rely on public awareness of diabetes mellitus as well as healthcare professional knowledge about the clinical presentation and diagnosis of paediatric diabetes mellitus and the accessibility to basic tests to confirm the diagnosis.

Malaysia is a low-middle income nation within the ASEAN region that has a well-supported public healthcare system and has undergone significant advances in infrastructure over the past several decades. In relation to childhood T1DM, several measures are already in place to facilitate a timely diagnosis. For instance, point of care testing is readily available for hospital-based healthcare professionals to diagnose hyperglycaemia, ketonaemia and acidosis in children suspected to have T1DM or DKA. Training of hospital doctors within the public sector on the updated versions of the National Clinical Practice Guidelines (CPG) on childhood T1DM take place with every iteration of the CPG. Educational sessions are also conducted by the National Paediatric Endocrine Society (Malaysian Paediatric Endocrine & Diabetes Group) for trainee paediatricians and family medicine doctors. Furthermore, the National Diabetes Institute of Malaysia and Diabetes Malaysia are instrumental in supporting people with diabetes and disseminating information about diabetes to the general public through their websites and magazines. The recently launched "Hello Type 1" by Action for Diabetes (A4D) for the Malaysian population is a website aimed at raising awareness about T1DM in the local language of Bahasa Melayu (32). Despite these efforts, rates of pDKA have remained high. However,

most of these measures have been in effect only over the last few years.

As such, future efforts should include research to understand the level of awareness of the general public and healthcare professionals about the clinical presentation of T1DM in children, in tandem with measures to raise public awareness about childhood diabetes and DKA, which has been shown to be beneficial in reducing rates of DKA in the UK, with the 4 T's campaign, and the Parma campaign in Italy (17,33). Regular continuous medical education about paediatric diabetes mellitus and DKA for primary care and hospital professionals may also help to improve diagnostic accuracy.

Study Limitations

A major limitation of this study is that it was retrospective, from a single centre and that it served an urban catchment area which is home to pockets of affluence and a highly educated population. This region is also home to a large concentration of paediatric endocrinologists and tertiary paediatric centres with dedicated PICUs which often receive referrals for severe DKA. These limitations may inflate the rates of severe DKA and PICU usage in this study. Future studies should include multiple centres from different regions of Malaysia, so that regional differences, risk factors and trends may be evaluated.

Conclusion

In summary, this study demonstrated that the incidence of pDKA at initial diagnosis of T1DM in Malaysian children has remained high over the 11-year study period. Severe DKA rates comprise a significant burden of the cases and has not reduced over the 11 years. Age >5 years and misdiagnosis emerged as two predictors of pDKA, of which misdiagnosis is a modifiable risk factor. Measures to reduce DKA rates need to focus on raising public awareness and physician awareness about T1DM and DKA in children. Future research should gather data on relevant socioeconomic factors which could influence a DKA presentation. The data should also be from multiple centres or a national registry to determine the true national rate of pDKA and to compare regional differences. This data could assist in developing needs-based strategies to curb the rates of DKA throughout the nation by implementing cost-effective methods for resource allocation.

Ethics

Ethics Committee Approval: This study was approved by the Universiti Malaya Medical Centre Institutional Ethics Board MREC ID no: 2019325-7251, date: 29.04.2019.

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Meenal Mavinkurve, Muhammad Yazid Bin Jalaludin, Nurshadia Samingan, Azriyanti Anuar Zaini, Concept: Azriyanti Anuar Zaini, Design: Meenal Mavinkurve, Nurul Hanis Ramzi, Azriyanti Anuar Zaini, Data Collection or Processing: Meenal Mavinkurve, Azriyanti Anuar Zaini, Analysis or Interpretation: Nurul Hanis Ramzi, Muhammad Yazid Bin Jalaludin, Azriyanti Anuar Zaini, Literature Search: Meenal Mavinkurve, Muhammad Yazid Bin Jalaludin, Azriyanti Anuar Zaini, Writing: Meenal Mavinkurve, Nurul Hanis Ramzi, Muhammad Yazid Bin Jalaludin, Nurshadia Samingan, Azriyanti Anuar Zaini.

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References

1. Maahs DM, Hermann JM, Holman N, Foster NC, Kapellen TM, Allgrove J, Schatz DA, Hofer SE, Campbell F, Steigleder-Schweiger C, Beck RW, Warner JT, Holl RW; National Paediatric Diabetes Audit and the Royal College of Paediatrics and Child Health, the DPV Initiative, and the T1D Exchange Clinic Network. Rates of diabetic ketoacidosis: international comparison with 49,859 pediatric patients with type 1 diabetes from England, Wales, the U.S., Austria, and Germany. Diabetes Care. 2015;38:1876-1882. Epub 2015 Aug 17
2. Gomber A, Ward ZJ, Ross C, Owais M, Mita C, Yeh JM, Reddy CL, Atun R. Variation in the incidence of type 1 diabetes mellitus in children and adolescents by world region and country income group: A scoping review. PLOS Glob Public Health. 2022;2:e0001099.
3. International Diabetes Federation. IDF Diabetes Atlas [Internet]. 9th ed. Brussels, Belgium: International Diabetes Federation; 2019 [cited 2020 Jul 15]. Available from: https://diabetesatlas.org/en/resources/
4. Jaruratanasirikul S, Thammaratchuchai S, Sriplung H. Trends of childhood diabetes in Southern Thailand: 20-year experience in a tertiary medical center. World J Pediatr. 2017;13:566-570. Epub 2017 Oct 20
5. IDF Diabetes Atlas 10th edition [Internet]. Available from: www.diabetesatlas.org
6. Pulungan AB, Fadiana G, Annisa D. Type 1 diabetes mellitus in children: experience in Indonesia. Clin Pediatr Endocrinol. 2021;30:11-18. Epub 2021 Jan 5
7. Tung JY, Kwan EY, But BW, Wong WH, Fu AC, Pang G, Tsang JW, Yau HC, Belaramani K, Wong LM, Wong SM, Lo P, Ng KL, Yeung WK, Chan KT, Chan AM, Wong SW, Tay MK, Chung J, Lee CY, Lam YY, Cheung PT. Increasing incidence of type 1 diabetes among Hong Kong children and adolescents: The Hong Kong Childhood Diabetes Registry 2008 to 2017. Pediatr Diabetes. 2020;21:713-719. Epub 2020 Apr 29
8. Fuziah MZ, Hong JY, Zanariah H, Harun F, Chan SP, Rokiah P, Wu LL, Rahmah R, Jamaiyah H, Geeta A, Chen WS, Adam B. A national

database on children and adolescent with diabetes (e-DiCARE): results from April 2006 to June 2007. Med J Malaysia. 2008;63(Suppl C):37-40.

9. Hong J, Jalaludin MY, Mohamad Adam B, Fuziah MZ, Wu LL, Rasat R, Fatimah H, Premaa S, Ponnudurai U, Jamaiyah H. Diabetic ketoacidosis at diagnosis of type 1 diabetes mellitus in Malaysian children and adolescents. Malays Fam Physician. 2015;10:11-18.
10. Poovazhagi V. Risk factors for mortality in children with diabetic keto acidosis from developing countries. World J Diabetes. 2014;5:932-938.
11. Roche EF. The National Paediatric Diabetes Register and its impact on healthcare. Ir Med J. 2014;107:197-198.
12. Hekkala A, Knip M, Veijola R. Ketoacidosis at diagnosis of type 1 diabetes in children in northern Finland: temporal changes over 20 years. Diabetes Care. 2007;30:861-866.
13. Schober E, Rami B, Waldhoer T; Austrian Diabetes Incidence Study Group. Diabetic ketoacidosis at diagnosis in Austrian children in 1989-2008: a population-based analysis. Diabetologia. 2010;53:1057-1061. Epub 2010 Mar 7
14. Usher-Smith JA, Thompson M, Ercole A, Walter FM. Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in children: a systematic review. Diabetologia. 2012;55:2878-2894. Epub 2012 Aug 30
15. Usher-Smith JA, Thompson MJ, Sharp SJ, Walter FM. Factors associated with the presence of diabetic ketoacidosis at diagnosis of diabetes in children and young adults: a systematic review. BMJ. 2011;343:d4092.
16. Mavinkurve M, Jalaludin MY, Chan EWL, Noordin M, Samingan N, Leong A, Zaini AA. Is Misdiagnosis of Type 1 Diabetes Mellitus in Malaysian Children a Common Phenomenon? Front Endocrinol (Lausanne). 2021;12:606018.
17. Vanelli M, Chiari G, Ghizzoni L, Costi G, Giacalone T, Chiarelli F. Effectiveness of a prevention program for diabetic ketoacidosis in children. An 8-year study in schools and private practices. Diabetes Care. 1999;22:7-9.
18. Jefferies C, Cutfield SW, Derraik JG, Bhagvandas J, Albert BB, Hofman PL, Gunn AJ, Cutfield WS. 15-year incidence of diabetic ketoacidosis at onset of type 1 diabetes in children from a regional setting (Auckland, New Zealand). Sci Rep. 2015;5:10358.
19. Dhatariya KK, Parsekar K, Skedgel C, Datta V, Hill P, Fordham R. The cost of treating diabetic ketoacidosis in an adolescent population in the UK: a national survey of hospital resource use. Diabet Med. 2019;36:982-987. Epub 2019 Jan 23
20. Kalla Vyas A, Oud L. Temporal patterns of hospitalizations for diabetic ketoacidosis in children and adolescents. PLoS One. 2021;16:e0245012.
21. de Onis M, Lobstein T. Defining obesity risk status in the general childhood population: which cut-offs should we use? Int J Pediatr Obes. 2010;5:458-460. Epub 2010 Mar 17
22. Roche EF, McKenna A, Ryder K, Brennan A, O'Regan M, Hoey H. The incidence of childhood type 1 diabetes in Ireland and the National Childhood Diabetes Register. Ir Med J. 2014;107:278-281.
23. Hekkala A, Reunanen A, Koski M, Knip M, Veijola R; Finnish Pediatric Diabetes Register. Age-related differences in the frequency of ketoacidosis at diagnosis of type 1 diabetes in children and adolescents. Diabetes Care. 2010;33:1500-1502. Epub 2010 Apr 22
24. Zucchini S, Scaramuzza AE, Bonfanti R, Buono P, Cardella F, Cauvin V, Cherubini V, Chiari G, d'Annunzio G, Frongia AP, Iafusco D, Maltoni G, Patera IP, Toni S, Tumini S, Rabbone I; Diabetes Study Group of The Italian Society For Pediatric Endocrinology And Diabetology. A Multicenter Retrospective Survey regarding Diabetic Ketoacidosis Management in Italian Children with Type 1 Diabetes. J Diabetes Res. 2016;2016:5719470. Epub 2015 Nov 15
25. Rittiphairoj T, Owais M, Ward ZJ, Reddy CL, Yeh JM, Atun R. Incidence and prevalence of type 1 diabetes and diabetic ketoacidosis in children and adolescents (0-19 years) in Thailand (2015-2020): A nationwide population-based study. Lancet Reg Health West Pac. 2022;21:100392.
26. Pulungan AB, RL Batubara J, Tridjaja B, Soesanti F, Fadiana G, Annisa D, Mada SI. Profile of Diabetic Ketoacidosis in Children with Diabetes Mellitus in a Tertiary Care Hospital in Jakarta: A Retrospective Study. Acta Sci Paediatr. 2019;2:24-29. Available from: https://actascientific.com/ASPE/pdf/ASPE-02-0052.pdf
27. Glaser N, Fritsch M, Priyambada L, Rewers A, Cherubini V, Estrada S, Wolfsdorf JI, Codner E. ISPAD clinical practice consensus guidelines 2022: Diabetic ketoacidosis and hyperglycemic hyperosmolar state. Pediatr Diabetes. 2022;23:835-856.
28. Dabelea D, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, Imperatore G, D'Agostino RB Jr, Mayer-Davis EJ, Pihoker C; SEARCH for Diabetes in Youth Study Group. Trends in the prevalence of ketoacidosis at diabetes diagnosis: the SEARCH for diabetes in youth study. Pediatrics. 2014;133:938-945. Epub 2014 Mar 31
29. Peng W, Yuan J, Chiavaroli V, Dong G, Huang K, Wu W, Ullah R, Jin B, Lin H, Derraik JGB, Fu J. 10-Year Incidence of Diabetic Ketoacidosis at Type 1 Diabetes Diagnosis in Children Aged Less Than 16 Years From a Large Regional Center (Hangzhou, China). Front Endocrinol (Lausanne). 2021;12:653519.
30. Cherubini V, Skrami E, Ferrito L, Zucchini S, Scaramuzza A, Bonfanti R, Buono P, Cardella F, Cauvin V, Chiari G, D Annunzio G, Frongia AP, Iafusco D, Patera IP, Toni S, Tumini S, Rabbone I, Lombardo F, Carle F, Gesuita R; Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology (ISPED). High frequency of diabetic ketoacidosis at diagnosis of type 1 diabetes in Italian children: a nationwide longitudinal study, 2004-2013. Sci Rep. 2016;6:38844.
31. Gunn ER, Albert BB, Hofman PL, Cutfield WS, Gunn AJ, Jefferies CA; Starbase Diabetes Working Group, Paediatric Diabetes Service, Starship Children's Hospital, Auckland, New Zealand. Pathways to reduce diabetic ketoacidosis with new onset type 1 diabetes: Evidence from a regional pediatric diabetes center: Auckland, New Zealand, 2010 to 2014. Pediatr Diabetes. 2017;18:553-558. Epub 2016 Oct 11
32. Hello Type 1. [Internet]. [Cited 2023 Sep 26]. Available from: https://hellotype1.com/en
33. Holder M, Ehehalt S. Significant reduction of ketoacidosis at diabetes onset in children and adolescents with type 1 diabetes-The Stuttgart Diabetes Awareness Campaign, Germany. Pediatr Diabetes. 2020;21:1227-1231. Epub 2020 Jul 30

ORIGINAL ARTICLE

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The Relationship Between Sleep Quality, Sleep Duration, Social Jet Lag and Obesity in Adolescents

Funda Yıldız[1], Melike Zeynep Tuğrul Aksakal[1], Raif Yıldız[2], Firdevs Baş[3]

[1]İstanbul University, Institute of Child Health, Department of Pediatric Basic Sciences, Clinic of Adolescent Medicine, İstanbul, Turkey
[2]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Pediatric Emergency, İstanbul, Turkey
[3]İstanbul University, İstanbul Faculty of Medicine, Department of Pediatrics, Clinic of Pediatric Endocrinology, İstanbul, Turkey

What is already known on this topic?

Previous studies have shown a relationship between obesity and sleep quality and sleep duration, but the issue of social jet lag (SJL), which represents shifts in circadian rhythm, and its effects on adolescents are still being investigated.

What this study adds?

This study shows a correlation between SJL and body mass index. Sleep history should be a part of the anamnesis in routine outpatient clinic examinations of overweight and obese adolescents which may aid the clinical management of the condition.

Abstract

Objective: The frequency of obesity and poor sleep quality among adolescents is increasing and causes many chronic problems. The objective was to investigate the correlation between body mass index (BMI), sleep quality, sleep duration and social jet lag (SJL) among adolescents.

Methods: This study was cross-sectional. A cohort of 416 adolescents, ranging in age from 12 to 18 years participated in the study. Adolescents were divided into three groups according to BMI standard deviation score (SDS): adolescents with normal weight, adolescents with overweight and adolescents with obesity. The Pittsburgh Sleep Quality Index (PSQI) questionnaire was used to determine the sleep quality of the adolescents. The calculation of SJL and sleep-corrected SJL was performed.

Results: The mean age of the adolescents was 15.0 ± 2.9 years. There were 222 males (53.4%). SJL and PSQI scores were significantly higher in the adolescents with obesity compared to the adolescents with normal weight and overweight ($p < 0.001$). An analysis of the relationship between the PSQI and BMI SDS revealed a significant positive correlation ($r = 0.667$; $p < 0.001$).

Conclusion: Adolescents with obesity have poorer sleep quality and a longer duration of SJL compared to adolescents with normal-weight. Moreover, increased SJL was linked to an increase in BMI. Maintaining good sleep quality and reducing SJL may help reduce the risk of obesity.

Keywords: Adolescents, sleep quality, social jet lag, obesity

Introduction

Sleep disorders are linked to disabilities, dangerous behavior, depression, morbidity and even mortality. According to research, children who sleep less are more prone to become overweight and obese later in life. Adolescents are at a significant risk of developing sleep disorders, and severe sleep deprivation is related with long-term consequences (1). The relationship between sleep disorders and adolescent obesity is not entirely understood. Trends in sleep problems parallel trends in obesity. Adolescent obesity may result

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Address for Correspondence: Funda Yıldız MD, İstanbul University, Institute of Child Health, Department of Pediatric Basic Sciences, Clinic of Adolescent Medicine, İstanbul, Turkey
E-mail: dr.fundayildiz@gmail.com **ORCID:** orcid.org/0000-0002-1008-4848

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in significant health issues. These conditions may include diabetes, hypertension, cardiovascular disease, respiratory illnesses, and musculoskeletal disorders (2). The intricate nature of the correlation between sleep duration and body weight is because both sleep and appetite are regulated by the daily circadian rhythm (3). Biologically, a lack of sleep alters the hormonal processes that control hunger, resulting in decreased levels of leptin and heightened levels of ghrelin. This inevitably leads to an increased consumption of food. Night-eating syndrome may arise due to sleep deprivation (4). During this condition, individuals display a tendency to consume food excessively and without inhibition upon awakening during the night. Overconsumption of calories can heighten the probability of getting obese. Insufficient sleep duration is significantly associated with adverse changes in body mass index (BMI) among infants, children, and adolescents, according to a meta-analysis (5). The quality of sleep is determined by the effectiveness and depth of an individual's sleep during their period of rest. A range of methodologies is employed by researchers in order to assess the quality of sleep. The Pittsburgh Sleep Quality Index (PSQI) is a widely acknowledged and scientifically proven method for assessing the quality of sleep (6). Studies have shown a correlation between obesity and the quality of sleep. Social jet lag (SJL) is defined as the discrepancy between the sleep timing imposed by external/social obligations, such as work or school days, and free days. Sleep deprivation occurs by waking up early on weekdays and going to bed late at night, and attempts are made to compensate for this by waking up late and sleeping too much at the weekend. This causes a discrepancy between biological and social circadian rhythms. SJL refers to a persistent and detrimental pattern of sleep disruption and inconsistent sleep. This sleep pattern can result in metabolic, physiological, and psychological complications (7). Recent findings indicate a correlation between SJL and obesity, suggesting that children who undergo SJL are at a higher risk of developing obesity (8). Nevertheless, the correlation between poor sleeping habits and obesity among adolescents remains poorly comprehended. The aim of this research was to examine the correlation between BMI, sleep quality, SJL status, and sleep duration among adolescents.

Methods

Study Participants and Procedure

This study was cross-sectional. The study protocol was approved by the İstinye University Human Research Ethics Committee (meeting number: 2023-09; protocol number: 23-238, date: 06.11.2023). The study included adolescents between the ages of 12 and 18 years who were admitted to a tertiary hospital without any diagnosis of psychiatric disorder, chronic disease or drug use and who agreed to participate in the study. Both written and verbal consent was obtained from the adolescents included in the study and their parents. The adolescents' age, weight, height, and BMI at the time of admission were recorded and they were asked to fill out a PSQI questionnaire, and their sleep-wake times on school days and weekends were noted.

Measures and Sleep Assesment

During the examinations of patients in the outpatient clinic, their height was measured using a Harpenden stadiometer (Holtain Ltd., Crymych, UK), and their weight was measured using a standard electronic scale (Beurer brand) that can detect changes of as little as 100 grams. BMI was calculated as weight (in kg) over the square of height (in meters) and converted to BMI standard deviation score (BMI SDS) using the Turkish national data. Adolescents were divided into three groups according to BMI: adolescents with normal weight; adolescent with overweight; and adolescent with obesity. The adolescents with obesity group was defined as BMI ≥95th percentile, the adolescents with overweight group was defined as BMI 94.9-85th percentile, and the adolescents with normal weight group was defined as BMI 5-84.9th percentile (9).

The PSQI questionnaire was used to determine the sleep quality in adolescents. The validity and reliability study for Turkish children was conducted by Ağargün et al. (10) PSQI is a 19-question survey with seven components (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping pills, daytime dysfunction). If the total PSQI score is five or less, it indicates good sleep quality. If it is more than five, it indicates poor sleep quality. Moreover, as the PSQI score increases, sleep quality worsens. In addition, to determine the average number of hours the adolescent sleeps the participant was asked exactly when he/she sleeps and at what time he/she wakes up in the morning, and then the duration of sleep was calculated. If the adolescent had a nap during the day, this was added. Both school days and free days were assessed using the same protocol. In order to calculate SJL and sleep-corrected SJL (SJLsc), as previously described (11,12). SJL is found by the absolute value of the difference between the middle hour of sleep on free days (midsleep on free days) and the middle hours of sleep on school days (midsleep of school days). The midpoint of sleep is the time between night sleep start time and morning wake-up time. Half of the weekly mean sleep duration is added to both the sleep start time on free days and the sleep start time on school

days, and then the values found are subtracted from each other, and the absolute difference is the SJLsc.

Statistical Analysis

The variables were analyzed using Statistical Package for the Social Sciences, version 25.0 (IBM Corp., Armonk, New York, USA) and Medcalc 14 (Acacialaan 22, B-8400 Ostend, Belgium) software. The assessment of whether the data followed a normal distribution was conducted using the Shapiro-Wilk-Francia test, while Levene's test was used to determine the homogeneity of variance. We compared two groups using Monte Carlo simulation outcomes and quantitative variables. We also used Monte Carlo simulation results in conjunction with the Kruskal-Wallis H test, a non-parametric test, to compare more than two groups of quantitative variables. Post-hoc analysis was subsequently conducted using Dunn's test. We used the Monte Carlo simulation method to examine the Pearson's chi-square test and the Fisher's Freeman Halton test in order to compare categorical variables. The correlation between two quantitative variables was assessed using the Spearman rho test. The research investigated the sensitivity, specificity, positive predictive ratio (positive predictive, positive predictivity), and negative predictive ratio (negative predictive, negative predictivity) in relation to the SJL variables-calculated cut-off values that separated the classifications. Analysis of the receiver operating curve analysis was performed to quantify the PSQI outcome variable and the actual classification. Categorical variables are denoted as n (percent), whereas quantitative variables were represented as median (minimum-maximum). Variables were analyzed with a confidence level of 95% and a $p < 0.05$ was deemed to indicate statistical significance.

Results

The study group included 416 adolescents with a mean age of 15.0 ± 2.9 years, of whom 222 (53.4%) were males. Of the 416 adolescents, 130 (31%) were normal weight, 151 (36%) were overweight and 135 (33%) were obese. Alarge part of (87%) both males and females rated their overall health as good or very good. Only 97 (23.3%) of all adolescents had good sleep quality. The average weekly sleep duration of all adolescents was 6.78±0.67 hours. The difference in total PSQI scores between the groups was significant ($p < 0.001$). The adolescents with obesity had a significantly higher median PSQI total score compared to both the adolescents with normal weight and overweight ($p < 0.001$). In addition, the adolescents with overweight group had a significantly higher median PSQI total score ($p = 0.001$) than the adolescents with normal weight group. There was also a significant difference in SJL between the adolescents with obesity, and the owerweight and normal weight groups ($p < 0.001$). The average duration of sleep per week was considerably shorter in the adolescents with obesity group in comparison to both the normal weight and overweight groups ($p < 0.001$). A significant correlation ($p < 0.001$) was observed between the groups and the daytime dysfunction score. Daytime dysfunction score I was prevalent at a rate of 61.5% in the adolescents with normal weight group, 8.6% in the adolescents with overweight group, and 1.5% in the adolescents with obesity group ($p < 0.001$). Daytime dysfunction, as defined in entry 7 of the PQSI, is a cluster of symptoms arising from poor sleep quality or other conditions, critical for daily activities and operational efficiency, especially high-precision tasks. and as the score increases, daytime dysfunction increases. Furthermore, the daytime dysfunction score 1 was significantly more prevalent in the adolescents with overweight group compared to the adolescents with obesity group ($p = 0.007$). In addition, a significant difference was found in the daytime dysfunction score 2 prevalence rates among the groups: the adolescents with overweight, with obesity and with normal weight (70.9% vs 53.3% vs 23.8%, respectively; $p < 0.001$) (Table 1). The prevalence of daytime dysfunction score 3 was significantly greater in the adolescents with obesity group (45.2%) compared to the overweight group (20.5%) ($p < 0.001$) and the normal weight group (0.8%) ($p < 0.001$). An examination of the connection between PSQI and BMI SDS revealed a moderate positive correlation ($r = 0.667$; $p < 0.001$) (Table 2). The groups did not show any significant differences when analyzed for gender distribution ($p = 0.472$), habitual sleep efficiency score ($p = 0.127$), and sleep medication use score ($p = 0.095$). SJL and SJLsc of > 1 hour was assessed in tems of poor sleep quality (PSQI score > 5) with a sensitivity of 74.6%, specificity of 88.7%, positive predictive value rate of 95.6%, and negative predictive value rate of 51.5%. The predictive power for optimal cut-off > 1 hour was very good ($p < 0.001$) (Table 3).

Discussion

The results of this cross-sectional study suggested that adolescents with obesity have poorer sleep quality, have a longer period of SJL, and have a shorter duration of sleep per week compared to adolescents with normal weight. In addition, there was a connection observed between SJL and a decrease in the quality of sleep. Furthermore, a moderate correlation was found between PSQI score and BMI. Optimizing sleep quality is an important objective for adolescents in general. The decline in sleep quality has been

Table 1. Characteristic features of adolescents and relationship between PSQI, sleep habits and the adolescents' BMI

	Total, n (%) (n = 416)	Normal weight (n = 130)	Overweight (n = 151)	Obese (n = 135)	p
	Med (min/max) or n (%)	Med (min/max) or n (%)	Med (min/max) or n (%)	Med (min/max) or n (%)	
Age (decimal)	15.06 (12.28/17.97)	14.55 (12.28/17.82)	15.05 (12.38/17.82)	15.44 (12.38/17.97)[A]	**0.006**[k]
Gender (female)	194 (46.6)	61 (46.9)	65 (43)	68 (50.4)	0.472[c]
BMI	25.02 (16.49/32.6)	-	-	-	-
BMI SDS	1.18 (-1.16/2.57)	-	-	-	-
PSQI ≤5 (Good Sleep Quality)	97 (23.3)	96 (73.8)	1 (0.7)[A]	-	**< 0.001**[c]
PSQI total score	7 (1/14)	4 (1/12)	7 (4/12)[A]	9 (5/14)[A, B]	**< 0.001**[k]
Subjective sleep quality score					**< 0.001**[ff]
0	82 (19.7)	81 (62.3)	1 (0.7)[B]	-	
1	199 (47.8)	47 (36.2)	102 (67.5)[A]	50 (37)[B]	
2	131 (31.5)	1 (0.8)	48 (31.8)[A]	82 (60.7)[A, B]	
3	4 (1)	1 (0.8)	-	3 (2.2)	
Sleep latency score					**< 0.001**[ff]
0	8 (1.9)	7 (5.4)	-	1 (0.7)[A]	
1	145 (34.9)	84 (64.6)	37 (24.5)[A]	24 (17.8)[A]	
2	163 (39.2)	32 (24.6)	75 (49.7)[A]	56 (41.5)[A]	
3	100 (24)	7 (5.4)	39 (25.8)[A]	54 (40)[A, B]	
Sleep duration score					**< 0.001**[ff]
0	115 (27.6)	93 (71.5)	15 (9.9)[A]	7 (5.2)[A]	
1	259 (62.3)	37 (28.5)	123 (81.5)[A]	99 (73.3)[A]	
2	42 (10.1)	-	13 (8.6)	29 (21.5)[B]	
Habitual sleep efficiency score					0.127[ff]
0	407 (97.8)	130 (100)	145 (96)	132 (97.8)	
1	8 (1.9)	-	5 (3.3)	3 (2.2)	
2	1 (0.2)	-	1 (0.7)	-	
Sleep disturbance score					**< 0.001**[ff]
0	11 (2.6)	11 (8.5)	-	-	
1	386 (92.8)	116 (89.2)	148 (98)[A]	122 (90.4)[B]	
2	19 (4.6)	3 (2.3)	3 (2)	13 (9.6)[A, B]	
Sleep medication use score					0.095[ff]
0	408 (98.1)	130 (100)	147 (97.4)	131 (97)	
1	6 (1.4)	-	2 (1.3)	4 (3)	
2	2 (0.5)	-	2 (1.3)	-	
Daytime dysfunction score					**< 0.001**[c]
0	18 (4.3)	18 (13.8)	-	-	
1	95 (22.8)	80 (61.5)	13 (8.6)[A]	2 (1.5)[A, B]	
2	210 (50.5)	31 (23.8)	107 (70.9)[A]	72 (53.3)[A, B]	
3	93 (22.4)	1 (0.8)	31 (20.5)[A]	61 (45.2)[A, B]	
Social jet lag	1.25 (0/4)	0.5 (0/2.25)	1.5 (0.5/3)[A]	2 (0.5/4)[A, B]	**< 0.001**[k]
Sleep corrected social jet lag	1 (0/4)	0.5 (0/2)	1.5 (0/3)[A]	1.5 (0/4)[A]	**< 0.001**[k]
Midpoint of sleep on school days	3:45 (2:00/4:45)	3:30 (2:00-4:30)	3:45 (2:00-4:45)[A]	4:00 (2:00-4:45)[A, B]	**< 0.001**[k]
Midpoint of sleep on free days	5:07 (3:00/7:30)	4:00 (3:00-6:00)	5:15 (3:00-7:00)[A]	5:30 (4:00-7:30)[A, B]	**< 0.001**[k]
Average weekly sleep duration	6.45 (4.3/8.45)	7.3 (6.15/8.45)	6.4 (5.25/8)[A]	6.3 (4.3/7.45)[A, B]	**< 0.001**[k]

[k]: Kruskal-Wallis H test (Monte Carlo); post-hoc: Dunn's test, [c]: Pearson's chi-square test (Monte Carlo); post-hoc: Benjamin-Hochberg test.
[ff]: Fisher's Freeman Halton test (Monte Carlo); post-hoc: Benjamin-Hochberg test.
[A]: Indicates significance compared to the 'normal weight' group. [B]: Indicates significance compared to the 'overweight' group.
Med: median, Min: minimum, Max: maximum, BMI: body mass index, SDS: standard deviation score, PSQI: Pittsburgh Sleep Quality Index

Table 2. Correlation analysis between PSQI, sleep habits and BMI, BMI SDS, and age

	BMI		BMI SDS		Age (decimal)		BMI*		BMI SDS*		BMI′		BMI SDS′		BMI‴		BMI SDS‴	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
PSQI Total score	0.623	**< 0.001**	0.667	**< 0.001**	0.145	**0.003**	0.597	**< 0.001**	0.655	**< 0.001**	0.637	**< 0.001**	0.661	**< 0.001**	0.636	**< 0.001**	0.654	**< 0.001**
Subjective sleep quality score	0.604	**< 0.001**	0.622	**< 0.001**	0.128	**0.009**	0.572	**< 0.001**	0.593	**< 0.001**	0.589	**< 0.001**	0.600	**< 0.001**	0.591	**< 0.001**	0.593	**< 0.001**
Sleep latency score	0.337	**< 0.001**	0.418	**< 0.001**	0.01	0.845	0.399	**< 0.001**	0.409	**< 0.001**	0.362	**< 0.001**	0.404	**< 0.001**	0.387	**< 0.001**	0.406	**< 0.001**
Sleep duration score	0.575	**< 0.001**	0.565	**< 0.001**	0.197	**< 0.001**	0.507	**< 0.001**	0.530	**< 0.001**	0.541	**< 0.001**	0.544	**< 0.001**	0.516	**< 0.001**	0.532	**< 0.001**
Habitual sleep efficiency score	0.025	0.609	0.077	0.117	-0.088	0.073	0.074	0.130	0.085	0.084	0.048	0.332	0.067	0.174	0.092	0.061	0.082	0.097
Sleep disturbance score	0.192	**< 0.001**	0.234	**< 0.001**	0.023	0.633	0.184	**< 0.001**	0.212	**< 0.001**	0.196	**< 0.001**	0.210	**< 0.001**	0.203	**< 0.001**	0.209	**< 0.001**
Sleep medication use score	0.015	0.758	0.062	0.209	-0.02	0.742	0.013	0.794	0.053	0.282	0.019	0.701	0.047	0.343	0.028	0.573	0.050	0.313
Daytime dysfunction score	0.614	**< 0.001**	0.631	**< 0.001**	0.192	**< 0.001**	0.584	**< 0.001**	0.641	**< 0.001**	0.641	**< 0.001**	0.649	**< 0.001**	0.623	**< 0.001**	0.639	**< 0.001**
Social jet lag	0.573	**< 0.001**	0.582	**< 0.001**	0.179	**< 0.001**	0.530	**< 0.001**	0.559	**< 0.001**	0.563	**< 0.001**	0.570	**< 0.001**	0.546	**< 0.001**	0.559	**< 0.001**
Sleep corrected social jet lag	0.522	**< 0.001**	0.541	**< 0.001**	0.152	**0.002**	0.504	**< 0.001**	0.535	**< 0.001**	0.531	**< 0.001**	0.545	**< 0.001**	0.519	**< 0.001**	0.535	**< 0.001**
Midpoint of sleep on school days	0.477	**< 0.001**	0.429	**< 0.001**	0.302	**< 0.001**	0.392	**< 0.001**	0.399	**< 0.001**	0.458	**< 0.001**	0.427	**< 0.001**	0.384	**< 0.001**	0.406	**< 0.001**
Midpoint of sleep on free days	0.687	**< 0.001**	0.674	**< 0.001**	0.296	**< 0.001**	0.637	**< 0.001**	0.670	**< 0.001**	0.686	**< 0.001**	0.678	**< 0.001**	0.648	**< 0.001**	0.672	**< 0.001**
Average weekly sleep duration	-0.57	**< 0.001**	-0.563	**< 0.001**	-0.171	**< 0.001**	-0.538	**< 0.001**	-0.556	**< 0.001**	-0.574	**< 0.001**	-0.570	**< 0.001**	-0.547	**< 0.001**	-0.558	**< 0.001**

Spearman rho correlation test, r: correlation coefficient, *: Partial correlation [controlling for age (decimal)], ′: Partial correlation (controlling for gender), ‴: Partial correlation [controlling for gender and age (decimal)].
BMI: body mass index, SDS: standard deviation score, PSQI: Pittsburgh Sleep Quality Index

Table 3. ROC analysis according to PSQI

Reference PSQI > 5 (Bed Sleep Quality)	Cut-off	Specificity	Sensitivity	-PV	+PV	AUC ± SE	p
All participants							
Social jet lag	> 1	88.7	74.6	51.5	95.6	0.886 ± 0.017	**< 0.001**
Sleep corrected social jet lag	> 1	96.9	51.7	37.9	98.2	0.837 ± 0.021	**< 0.001**
Male							
Social jet lag	> 1	90.6	77.5	55.8	96.3	0.901 ± 0.021	**< 0.001**
Sleep corrected social jet lag	> 1	54.7	95.9	80.6	87.1	0.851 ± 0.029	**< 0.001**
Female							
Social jet lag	> 1	86.4	71.3	46.9	94.7	0.867 ± 0.026	**< 0.001**
Sleep corrected social jet lag	> 1	97.7	50.7	36.8	98.7	0.821 ± 0.031	**< 0.001**
Normal weight							
Social jet lag	> 1	88.5	35.3	79.4	52.2	0.627 ± 0.060	**0.034**
Sleep corrected social jet lag	> 1	49.0	64.7	79.7	31	0.580 ± 0.058	0.170

Analysis Honley&Mc Nell-Youden index J.
ROC: receiver operating curve, AUC: area under the ROC curve, SE: standard error, +PV: positive predictive value, -PV: negative predictive value, PSQI: Pittsburgh Sleep Quality Index

specifically linked to compromised social function, decreased immunity, obesity, and poor performance in school (13). According to the National Sleep Foundation Sleep in America Poll, 75% of 12th graders reported getting less than eight hours of sleep a night. However, research generally shows that it is important for young people to get at least 8-9 hours of sleep a night (14). The optimal duration of sleep can vary between individuals and may be influenced by the age, and the physical and mental requirements of young individuals. A study examining the correlation between the amount of sleep and body fat levels in adolescents reported that decreased sleep duration was associated with increased body fat in adolescents. Furthermore, a direct correlation was found between decreased sleep duration and increased risk of obesity (15). We found that the adolescents who participated in our study had an average week night sleep duration of 6.63 hours which is below the recommended duration. Studies indicate that insufficient sleep time and poor sleep quality elevate the likelihood of developing obesity (16). In individuals with suboptimal sleep patterns, factors such as disturbances in hormonal control, heightened appetite, reduced energy expenditure, and an elevated propensity for weight gain may be operative. Insufficient sleep can heighten the inclination to consume larger quantities of food due to its impact on the appetite-regulating hormones, leptin and ghrelin. Insufficient sleep can result in reduced metabolic function, insulin resistance, and alterations in body composition (17,18). Studies indicate a correlation between SJL and obesity (19). SJL refers to the difference between weekday and weekend sleep hours. In other words, it is the habit of waking up early and staying awake until late on weekdays, going to sleep later, and sleeping longer on weekends. This change in sleep patterns can disrupt sleep patterns and affect the body's biological clock. Research has shown that individuals who experience more SJL generally have a higher BMI and are more prone to obesity (20). However, researchers have yet to determine the precise mechanism by which SJL affects obesity but it has been shown that SJL negatively affects sleep duration and sleep quality. Decreased sleep duration and quality can lead to disruption of hormonal balance, increased appetite, and an irregular metabolism (8). This increases the risk of obesity. Our investigation found a notable correlation between heightened SJL and elevated BMI, consistent with the existing literature. The quality of sleep can be influenced by different factors. Additional variables, including age, gender, lifestyle, and genetic predisposition can influence the quality of sleep (21). The association between BMI and PSQI score we found remained significant, even after controlling for age and gender variables.

Study Limitations

The participants' sleep-wake times were self-reported or reported by parents. If actigraphy could have been used, we would be able to obtain accurate results for sleep duration and SJL. In addition, food consumption records and physical activity scores were not included in this study. There is a bidirectional relationship between short sleep duration and obesity. We also found that even if the total weekly sleep duration was at normal values, adolescents' exposure to more SJL increased BMI. We believe that this bidirectional relationship will be useful both in outpatient

clinic administrations for the prevention of obesity and in sleep studies related to circadian rhythm.

Conclusion

This study demonstrated a relationship between inadequate sleep quality, increased exposure to SJL, and a higher BMI in adolescents. We predict that improving sleep quality during adolescence may have a protective effect in preventing obesity. We recommend that all adolescents, especially those with overweight and obesity, be questioned about their sleep habits during their routine outpatient clinic examinations and that their sleep history be a part of the anamnesis. However, additional study is required to develop suitable guidelines for sleep recommendations for adolescents.

Ethics

Ethics Committee Approval: The study protocol was approved by the İstinye University Human Research Ethics Committee (meeting number: 2023-09; protocol number: 23-238, date: 06.11.2023).

Informed Consent: Written informed consent was obtained from all patients.

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Footnotes

Authorship Contributions

Concept: Funda Yıldız, Melike Zeynep Tuğrul Aksakal, Firdevs Baş, Design: Funda Yıldız, Melike Zeynep Tuğrul Aksakal, Firdevs Baş, Data Collection or Processing: Funda Yıldız, Melike Zeynep Tuğrul Aksakal, Analysis or Interpretation: Funda Yıldız, Raif Yıldız, Firdevs Baş, Literature Search: Funda Yıldız, Melike Zeynep Tuğrul Aksakal, Raif Yıldız, Firdevs Baş, Writing: Funda Yıldız, Melike Zeynep Tuğrul Aksakal, Raif Yıldız, Firdevs Baş.

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References

1. Johnsen L, Bird JC, Salkovskis P, James AC, Stratford HJ, Sheaves B. Sleep disruption in adolescent inpatients: prevalence, associations with clinical outcomes, and clinician perspectives. J Sleep Res. 2023;3:e14056. Epub 2023 Oct 3
2. Ji X, Covington LB, Patterson F, Ji M, Brownlow JA. Associations between sleep and overweight/obesity in adolescents vary by race/ethnicity and socioeconomic status. J Adv Nurs. 2023;79:1970-1981. Epub 2022 Nov 28
3. Broussard JL, Van Cauter E. Disturbances of sleep and circadian rhythms: novel risk factors for obesity. Curr Opin Endocrinol Diabetes Obes. 2016;23:353-359.
4. Broussard JL, Kilkus JM, Delebecque F, Abraham V, Day A, Whitmore HR, Tasali E. Elevated ghrelin predicts food intake during experimental sleep restriction. Obesity (Silver Spring). 2016;24:132-138. Epub 2015 Oct 15
5. Deng X, He M, He D, Zhu Y, Zhang Z, Niu W. Sleep duration and obesity in children and adolescents: evidence from an updated and dose-response meta-analysis. Sleep Med. 2021;78:169-181. Epub 2020 Dec 29
6. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193-213.
7. Bouman EJ, Beulens JWJ, den Braver NR, Blom MT, Remmelzwaal S, Elders PJM, Rutters F. Social jet lag and (changes in) glycemic and metabolic control in people with type 2 diabetes. Obesity (Silver Spring). 2023;31:945-954. Epub 2023 Feb 28
8. Roenneberg T, Allebrandt KV, Merrow M, Vetter C. Social jetlag and obesity. Curr Biol. 2012;22:939-943. Epub 2012 May 10 Erratum in: Curr Biol. 2013;23:737.
9. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.
10. Ağargün MY, Kara H, Anlar Ö. The Validity and Reliability of the Pittsburgh Sleep Quality Index. Turkish J Psychiatry. 1996;7:107-111.
11. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23:497-509.
12. Jankowski KS. Social jet lag: Sleep-corrected formula. Chronobiol Int. 2017;34:531-535. Epub 2017 Mar 20
13. Sluggett L, Wagner SL, Harris RL. Sleep Duration and Obesity in Children and Adolescents. Can J Diabetes. 2019;43:146-152. Epub 2018 Jul 4
14. Paruthi S, Brooks LJ, D'Ambrosio C, Hall WA, Kotagal S, Lloyd RM, Malow BA, Maski K, Nichols C, Quan SF, Rosen CL, Troester MM, Wise MS. Recommended Amount of Sleep for Pediatric Populations: A Consensus Statement of the American Academy of Sleep Medicine. J Clin Sleep Med. 2016;12:785-786.
15. Calcaterra V, Rossi V, Tagi VM, Baldassarre P, Grazi R, Taranto S, Zuccotti G. Food Intake and Sleep Disorders in Children and Adolescents with Obesity. Nutrients. 2023;15:4736.
16. Rafique N. Short sleep duration is a novel independent risk factor for overweight and obesity. Saudi Med J. 2023;44:1160-1166.
17. Sharma S, Kavuru M. Sleep and metabolism: an overview. Int J Endocrinol. 2010;2010:270832. Epub 2010 Aug 2
18. Lin J, Jiang Y, Wang G, Meng M, Zhu Q, Mei H, Liu S, Jiang F. Associations of short sleep duration with appetite-regulating hormones and adipokines: A systematic review and meta-analysis. Obes Rev. 2020;21:e13051. Epub 2020 Jun 15
19. Malone SK, Zemel B, Compher C, Souders M, Chittams J, Thompson AL, Pack A, Lipman TH. Social jet lag, chronotype and body mass index in 14-17-year-old adolescents. Chronobiol Int. 2016;33:1255-1266. Epub 2016 Aug 11
20. Pompeia S, Panjeh S, Louzada FM, D'Almeida V, Hipolide DC, Cogo-Moreira H. Social jetlag is associated with adverse cardiometabolic latent traits in early adolescence: an observational study. Front Endocrinol (Lausanne). 2023;14:1085302.
21. Cespedes Feliciano EM, Rifas-Shiman SL, Quante M, Redline S, Oken E, Taveras EM. Chronotype, Social Jet Lag, and Cardiometabolic Risk Factors in Early Adolescence. JAMA Pediatr. 2019;173:1049-1057.

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Electrocardiographic Findings in Children Treated with Leuprolide Acetate for Precocious Puberty: Does it Cause Prolonged QT?

Esma Ebru Altun, Ayşe Yaşar, Fatma Dursun, Gülcan Seymen, Heves Kırmızıbekmez

University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Pediatric Endocrinology, İstanbul, Turkey

What is already known on this topic?

Gonadotropin releasing hormone (GnRH) analogues are the only drugs used in the treatment of central precocious puberty (CPP) in children. Increased cardiovascular events and especially a serious disorder of prolonged QT in some adult patient groups while receiving GnRH analogue treatment have been reported in recent studies. Prolonged QT and arrhythmias are life-threatening conditions and can be detected by electrocardiogram (ECG), which is a cheap, non-invasive, and easily accessible test. However, more evidence-based information is needed to recommend ECG before and during GnRH analogue treatment in children.

What this study adds?

In this study, no prolonged QT or other pathological electrophysiological findings were found in young girls, aged 5-11 years, receiving leuprolide acetate treatment due to CPP. No correlation was found between corrected QT values and age, treatment duration, total cumulative dose, and anthropometric data in this cohort. These results suggested no adverse effect of leuprolide acetate on cardiovascular adverse events.

Abstract

Objective: Central precocious puberty (CPP) is treated with long-acting gonadotropin releasing hormone (GnRH) analogues (GnRHa). Some adult patients undergoing GnRHa treatment experienced prolonged QT syndrome, which is associated with an increased risk of serious cardiac events, such as myocardial infarction, stroke, arrhythmias, and sudden cardiac death.

Methods: Seventy-four patients, aged between 5 and 11 years and diagnosed with CPP but with no other concomitant disease or medication use, underwent electrocardiogram (ECG) assessment. They had been receiving 3.75 mg leuprolide acetate (Lucrin® Depot) injections every 28 days for at least three months.

Results: The ECGs of all patients showed a corrected QT (QTc) interval within normal limits, consistent with the data for healthy Turkish children of the same age and gender. No other pathological physical examination or ECG findings were observed. Furthermore, there was no significant difference in QTc interval when adjusted for age, anthropometric data, or the duration or cumulative dose of the treatment.

Conclusion: The study found no correlation between QTc interval values and age, treatment duration, total cumulative dose, and anthropometric data. These findings suggest that cardiovascular adverse events associated with GnRHa treatment may be related to age and other underlying physiopathological conditions in adults rather than being directly due to the drug.

Keywords: Precocious puberty, leuprolide acetate, children, ECG, prolonged QT

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Address for Correspondence: Heves Kırmızıbekmez MD, University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Pediatric Endocrinology, İstanbul, Turkey
E-mail: heveskirmizibekmez@yahoo.com **ORCID:** orcid.org/0000-0002-8663-3452

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Introduction

Central precocious puberty (CPP) is the premature development of secondary sexual characteristics in girls before the age of 8 years and in boys before the age of 9 years due to the early maturation of the hypothalamic-pituitary-gonadal axis (1). The treatment of CPP involves the use of long-acting gonadotropin releasing hormone (GnRH) analogues (GnRHas), which paradoxically downregulate and subsequently suppress the HPG axis. These drugs have been used for many years (2). The aim of treatment for CPP is to preserve height potential, prevent early menarche, and address psychosocial issues. GnRH agonists are commonly used in the treatment of conditions such as prostate and breast cancer, endometriosis, and uterine fibroids in adults. It has been reported that adult patients undergoing GnRHa treatment may develop prolonged QT syndrome, which is linked to a higher risk of serious cardiac events, including myocardial infarction, stroke, arrhythmias, and sudden cardiac death. The elevated incidence of cardiovascular events in adult male patients undergoing androgen deprivation therapy for prostate cancer has been mainly attributed to the androgen deprivation. However, GnRH agonists have been found to be more strongly linked to cardiovascular events than other agents, such as GnRH antagonists, used for androgen deprivation. It has been suggested that GnRH agonists have a more significant impact on cardiovascular events beyond androgen deficiency (3,4,5,6).

The drug's prospectus reports these complications, but there is no evidence in the literature regarding their effects on women and children. Thus, the aim of this study was to investigate the effect of leuprolide acetate treatment on electrocardiogram (ECG) findings in children with CPP. Investigating the effects of this treatment on cardiac rhythm and corrected QT (QTc) interval is important to assess the safety of this drug in the pediatric population.

Methods

Girls aged between 5 and 11 years and diagnosed with CPP, were included in this prospective cross-sectional study. The study received approval from Ümraniye Training and Research Hospital Local Ethics Committee (approval number: B.10.1.TKH.4.34.H.GP.0.01/160, date: 15.05.2023). The authors have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals.

The decision for treatment was typically made on an individual basis, considering the patient's age at puberty onset, rate of progression of puberty, accelerated growth and bone age, hormone levels, ultrasonographic measurements, and the expectation of early menarche. The study included patients who had been receiving 3.75 mg leuprorelin acetate (Lucrin® Depot) injections every 28 days for at least three months. The birth weight, week of gestation at birth, nutritional status, and history of any previous or ongoing disease medication use were recorded. The study included patients who only used leuprolide acetate as GnRHa therapy and were still under treatment during the study period. Among these patients, patients who had any health problems other than early puberty, who used any other medications within the last three months (therapy for allergic diseases, attention disorders, epilepsy, psychopathologies, inflammatory diseases), and who did not agree to have an ECG at the baseline study visit were excluded. A repeat ECG was requested if it was not of sufficient quality due to artifacts, but those who did not have a repeat ECG were also excluded from the study.

All patients underwent a thorough physical examination, with particular attention paid to the cardiovascular system, and an ECG test at their first visit after being enrolled in the study. During this visit, the following parameters were recorded: age, duration of treatment (in months), cumulative dose of leuprolide acetate (calculated as total mg/kg), height, weight, and body mass index. Anthropometric data were recorded as standard deviation (SD) scores which were calculated using an online application (Child Metrics®) which uses reference data for Turkish children (7,8). The ECG results were evaluated by a pediatric cardiologist. The cardiac rhythm, heart rate, and QTc interval, calculated using the Bazett formula, were recorded (9). The primary focus of ECG analysis was the QTc interval, which is key for assessing the cardiac repolarization phase and potential arrhythmia risk (10). Correlation analyses were used to investigate potential relationships between the variables and ECG measurements. The QTc intervals of the patients were compared to the reference values of age- and gender-matched healthy Turkish children. The reference value for 5-8 year-old girls is 422 (382-465) milliseconds (ms) and for 8-12 year-old girls is 422 (377-486) ms (11).

Statistical Analysis

Statistical analysis was conducted using IBM Statistical Package for the Social Sciences, version 22 (IBM Inc., Armonk, NY, USA). The normality of the data was evaluated using the Shapiro-Wilk test. The results are presented as mean ± SD since the data was normally distributed. The independent-samples t-test was used to compare the independent groups. The Wilcoxon signed test was used to compare related samples. For correlation analyses, the

Pearson test was used for normally distributed variables. The level of significance for all analyses was set at $p < 0.05$.

Results

The study analyzed a cohort of 74 female patients, aged between 5 and 11 years, who were receiving leuprolide acetate. The mean age at the start of treatment was 7.58 ± 0.91, ranging 5.2-9.5 years, and the mean age at which an ECG was performed was 8.95 ± 1.17 years, ranging 5.5-11.0 years. The mean total duration of treatment before the ECG was 17.6 ± 10.5 months, with a minimum of 3 months and a maximum of 66 months, and the cumulative dose received was 58.4 ± 31.3 mg/m^2, ranging 10.04-186.7 mg/m^2.

The cardiology assessment showed no symptoms or pathological physical examination findings. Two patients had nonspecific ST-T changes. Among the 72 patients with normal ECG findings, 37 had respiratory sinus arrhythmia and two had only one atrial escape beat. The QTc interval was within normal limits in all ECGs. The mean QTc was 390 ± 10 ms, ranging from 360-430 ms, which is within normal limits and was not longer than the reference value for healthy Turkish children according to age and gender. Non-parametric tests revealed no difference between QT intervals before treatment and at the end of sixth month of the treatment in seven patients who were newly diagnosed during the study ($p = 0.753$). There was no significant difference between patients who received leuprolide acetate treatment for 18 months or more and those who received it for a shorter period. In addition, there was no significant difference between patients who received a leuprolide acetate cumulative dose of 2 mg/kg or more and those who received less (Table 1). Our analysis did not reveal any correlation between the QTc values and the patients' age, duration of treatment, cumulative dose, or anthropometric data, as shown in Table 2.

Discussion

This study evaluated ECG findings in young girls, aged 5-11 years, who were receiving leuprolide acetate treatment for CPP. No prolonged QT or other pathological electrophysiological findings were observed in any of the 74 patients. The absence of correlation between QTc values and age, treatment duration, total cumulative dose, and anthropometric data in our patients suggests that the adverse cardiovascular events previously reported in adults may be due to different underlying pathological mechanisms rather than any direct effect of the drug.

Recent reports have highlighted an increased risk of cardiovascular events, including prolonged QT syndrome, in some adult patients receiving GnRHa treatment. This has raised concerns about the safety of GnRHas, which are the only treatment agents used for CPP in children. Prolonged QT and arrhythmias may be detected by ECG, a cheap,

Table 1. The comparison of QTc intervals of patients on leuprolide acetate between short- and long-term users, and between higher- and lower-dose users

Duration of treatment			
	≥18 months (n = 37)	**< 18 months (n = 37)**	**p**
QTc interval (ms)	393 ± 18	395 ± 21	0.716
Cumulative dose of the GnRHa			
	≥2 mg/kg (n = 35)	**< 2 mg/kg (n = 39)**	**p**
QTc interval (ms)	391 ± 18	397 ± 21	0.200

QTc: corrected QT, GnRHa: gonadotropin releasing hormone analogues

Table 2. Correlations of treatment parameters and anthropometric measurements with corrected QT interval

QTc interval		
	r	**p**
Age	-0.064	0.602
Duration of treatment (months)	-0.090	0.463
Cumulative dose (mg/kg)	-0.068	0.577
Cumulative dose (mg/m^2)	-0.080	0.515
Weight SDS	-0.050	0.684
Height SDS	0.001	0.997
BMI SDS	-0.057	0.641

QTc: corrected QT, BMI: body mass index, SDS: standard deviation score

non-invasive, and easily accessible test. However, more information should be provided before recommending ECGs before and during GnRHa treatment in children.

Acquired prolonged QT syndrome can be caused by several major classes of drugs, with new ones continuing to be identified. A study from the United States reported antiarrhythmic drugs were responsible for 77% of cases (12). Other medications associated with prolonged QT include psychotropic drugs, gastrointestinal medications, antimicrobials, and tyrosine kinase inhibitors. Antimicrobial medications that prolong QT include macrolide antibiotics, fluoroquinolone antibiotics, and antifungal drugs (13). It has been reported that erythromycin led to a two-fold increase in risk of sudden cardiac death compared to nonusers (14). Painkillers (non-steroidal anti-inflammatory drugs, opioids, anticonvulsants, antidepressants, cannabinoids, and muscle relaxants), proton pump inhibitors, antiemetics, and diuretics are also reported to be causes of prolonged QT (15,16).

Antiarrhythmic drugs are used for cardiological indications and are followed by repeated ECGs under the supervision of a cardiologist. However, antimicrobial treatments, painkillers, and proton pump inhibitors are used without such precautions. Furthermore, there is no recommendation for cardiological evaluation before starting or during follow-up for GnRHas.

Waldner et al. (17) recently reported a study of 33 gender-diverse young people who were initiated on leuprolide acetate. The mean age of the cohort was 13.7 ± 2.1 years, and the mean post-leuprolide acetate QTc was 415 ± 27 ms (range 372-455). Only 24.2% of the patients had a borderline QTc (440-460 ms), and none had a prolonged QTc despite concomitant medications in twenty-two (66.7%).

The Pediatric Endocrine Society has issued guidelines regarding the potential risk of GnRHas. It is recommended to perform a screening ECG for patients who are on a medication known to cause QTc prolongation, have a personal history of congenital heart disease, arrhythmia, or long QT syndrome, have a family history of long QT syndrome or sudden cardiac death, and for those who experience symptoms of long QT syndrome, including syncope. It is recommended to perform a repeat ECG when the GnRHa dose has reached steady state in these groups. Patients should also be counseled about symptoms of arrhythmia, including palpitations and syncope. The authors conclude that further studies are necessary to investigate the risk of prolonged QT with GnRHa therapy in children and young adults (18).

Study Limitations

This was a single center study conducted in a limited number of patients. This was because of the exclusion of patients having any additional disease in addition to CCP or were on medications in addition to leuprolide acetate. The main limitation was the small number of patients who were newly diagnosed and underwent ECG before the treatment was started.

Conclusion

The results of this study showed no prolonged QT or any other ECG abnormality with short- or long-term exposure to GnRHa treatment, leuprolide acetate, in young girls with CPP.

Ethics

Ethics Committee Approval: Ethics approval to conduct this study was obtained from the Medical Ethics Committee of the University of Oldenburg (no: 2021-024, date: 11.02.2021).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Esma Ebru Altun, Ayşe Yaşar, Fatma Dursun, Gülcan Seymen, Heves Kırmızıbekmez, Concept: Esma Ebru Altun, Heves Kırmızıbekmez, Design: Esma Ebru Altun, Heves Kırmızıbekmez, Data Collection or Processing: Esma Ebru Altun, Ayşe Yaşar, Fatma Dursun, Gülcan Seymen, Heves Kırmızıbekmez, Analysis or Interpretation: Esma Ebru Altun, Ayşe Yaşar, Heves Kırmızıbekmez, Literature Search: Esma Ebru Altun, Ayşe Yaşar, Fatma Dursun, Gülcan Seymen, Heves Kırmızıbekmez, Writing: Esma Ebru Altun, Heves Kırmızıbekmez.

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References

1. Cheuiche AV, da Silveira LG, de Paula LCP, Lucena IRS, Silveiro SP. Diagnosis and management of precocious sexual maturation: an updated review. Eur J Pediatr. 2021;180:3073-3087. Epub 2021 Mar 21
2. Eugster EA. Treatment of Central Precocious Puberty. J Endocr Soc. 2019;3:965-972.

3. Abbasi D, Faiek S, Shetty S, Khan E. Shock From Twisting Peaks: A Rare Case of Recurrent Torsades de Pointes Secondary to Leuprolide-Induced Prolonged QT. Cureus. 2020;12:e9041.
4. Kim J, Freeman K, Ayala A, Mullen M, Sun Z, Rhee JW. Cardiovascular Impact of Androgen Deprivation Therapy: from Basic Biology to Clinical Practice. Curr Oncol Rep. 2023;25:965-977. Epub 2023 Jun 5
5. Olsson H, Petri N, Erichsen L, Malmberg A, Grundemar L. Effect of Degarelix, a Gonadotropin-Releasing Hormone Receptor Antagonist for the Treatment of Prostate Cancer, on Cardiac Repolarisation in a Randomised, Placebo and Active Comparator Controlled Thorough QT/QTc Trial in Healthy Men. Clin Drug Investig. 2017;37:873-879.
6. Albertsen PC, Klotz L, Tombal B, Grady J, Olesen TK, Nilsson J. Cardiovascular morbidity associated with gonadotropin releasing hormone agonists and an antagonist. Eur Urol. 2014;65:565-573. Epub 2013 Nov 1
7. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.
8. Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A Comprehensive Online Calculator for Pediatric Endocrinologists: ÇEDD Çözüm/TPEDS Metrics. J Clin Res Pediatr Endocrinol. 2017;9:182-184. Epub 2017 Apr 26
9. Dahlberg P, Diamant UB, Gilljam T, Rydberg A, Bergfeldt L. QT correction using Bazett's formula remains preferable in long QT syndrome type 1 and 2. Ann Noninvasive Electrocardiol. 2021;26:e12804. Epub 2020 Oct 18
10. Lester RM, Paglialunga S, Johnson IA. QT Assessment in Early Drug Development: The Long and the Short of It. Int J Mol Sci. 2019;20:1324.
11. Semizel E, Oztürk B, Bostan OM, Cil E, Ediz B. The effect of age and gender on the electrocardiogram in children. Cardiol Young. 2008;18:26-40. Epub 2007 Dec 20
12. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George AL Jr, Roden DM. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation. 2002;105:1943-1948.
13. Charles I Berul. Acquired long QT syndrome: Definitions, pathophysiology, and causes. Up To date (ed. Samuel Asirvatham); last updated Sep 21, 2022.
14. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. N Engl J Med. 2004;351:1089-1096.
15. Kallergis EM, Goudis CA, Simantirakis EN, Kochiadakis GE, Vardas PE. Mechanisms, risk factors, and management of acquired long QT syndrome: a comprehensive review. ScientificWorldJournal. 2012;2012:212178. Epub 2012 Apr 19
16. Klivinyi C, Bornemann-Cimenti H. Pain medication and long QT syndrome. Korean J Pain. 2018;31:3-9. Epub 2018 Jan 2
17. Waldner RC, Doulla M, Atallah J, Rathwell S, Grimbly C. Leuprolide Acetate and QTc Interval in Gender-Diverse Youth. Transgend Health. 2023;8:84-88.
18. Miller BS, Kamjob M; on behalf of the Drug and Therapeutics Committee. Risk of Prolonged QT Interval with Gonadotropin Releasing Hormone Agonists. Mclean, VA: Pediatric Endocrine Society, 2017.

ORIGINAL ARTICLE

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Molecular Genetic Diagnosis with Targeted Next Generation Sequencing in a Cohort of Turkish Osteogenesis Imperfecta Patients and their Genotype-phenotype Correlation

Samim Özen[1], Damla Gökşen[1], Ferda Evin[2], Esra Işık[3], Hüseyin Onay[4], Bilçağ Akgün[4], Aysun Ata[1], Tahir Atik[3], Füsun Düzcan[5], Ferda Özkınay[3], Şükran Darcan[1], Özgür Çoğulu[3]

[1]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, İzmir, Turkey
[2]Bakırçay University, Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology, İzmir, Turkey
[3]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Genetics, İzmir, Turkey
[4]Ege University Faculty of Medicine, Department of Medical Genetics, İzmir, Turkey
[5]Tınaztepe University Faculty of Medicine, Department of Medical Genetics, İzmir, Turkey

What is already known on this topic?

Variants in *COL1A1* and *COL1A2* genes, encoding type 1 collagen, are responsible for most of the etiology in osteogenesis imperfecta (OI). Molecular diagnosis is useful for early diagnosis, estimating the prognosis, determination of other individuals in the family and choice of treatment according to knowledge of variable responses to drugs.

What this study adds?

By using a targeted OI next-generation sequencing (NGS) panel *(COL1A1, COL1A2, IFITM5, SERPINF1, CRTAP, P3H1, PPIB, SERPINH1, FKBP10, SP7, BMP1, MBTPS2, PLOD2)*, the detection rate of disease causing variants was as high as 82.1% *(COL1A1, COL1A2, P3H1, SERPINF1, FKBP10)* in pediatric patients. We believe that targeted NGS was a valuable method for genetic diagnosis in pediatric patients with OI.

Abstract

Objective: Osteogenesis imperfecta (OI) consists of a group of phenotypically and genetically heterogeneous connective tissue disorders that share similar skeletal anomalies causing bone fragility and deformation. The aim was to investigate the molecular genetic etiology and determine the relationship between genotype and phenotype in OI patients using targeted next-generation sequencing (NGS).

Methods: A targeted NGS analysis panel (Illumina TruSight One) containing genes involved in collagen/bone synthesis was performed on the Illumina Nextseq550 platform in patients with a confirmed diagnosis of OI.

Results: Fifty-six patients (female/male: 25/31) from 46 different families were included. Consanguinity was noted in 15 (32.6%) families. Based on Sillence classification 18 (33.1%) were type 1 OI, 1 (1.7%) type 2, 26 (46.4%) type 3 and 11 (19.6%) type 4. Median body weight was -1.1 (-6.8, - 2.5) standard deviation scores (SDS), and height was -2.3 (-7.6, - 1.2) SDS. Bone deformity affected 30 (53.5%), while 31 (55.4%) were evaluated as mobile. Thirty-six (60.7%) had blue sclera, 13 (23.2%) had scoliosis, 12 (21.4%) had dentinogenesis imperfecta (DI), and 2 (3.6%) had hearing loss. Disease-causing variants in *COL1A1* and *COL1A2* were found in 24 (52.1%) and 6 (13%) families, respectively. In 8 (17.3%) of the remaining 16 (34.7%) families, the NGS panel revealed disease-causing

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Address for Correspondence: Ferda Evin MD, Bakırçay University, Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology and Diabetes, İzmir, Turkey
E-mail: ferdaevin88@gmail.com **ORCID:** orcid.org/0000-0001-7169-890X

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variants in three different genes (*FKBP10, SERPINF1*, and *P3H1*). Nine (23.6%) of the variants detected by NGS panel had not previously been reported and were also classified as pathogenic based on American College of Medical Genetics guidelines pathogenity scores. In ten (21.7%) families, a disease-related variant was not found in any of the 13 OI genes on the panel.
Conclusion: Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis. Furthermore, nine new variants were identified in known OI genes which were classified as pathogenic by standard guidelines.
Keywords: Osteogenesis imperfecta, next-generation sequencing, *COL1A1*, genetics

Introduction

Osteogenesis imperfecta (OI) is a hereditary disease of connective tissue characterized by increased bone fragility and multiple fractures (1,2,3). OI is a rare disorder with a frequency of 1/15,000-20,000. This generalized connective tissue disorder has an important effect on bone structure, leading to skeletal fragility and developmental delay. Clinical severity varies between the types of OI and additional features such as dentinogenesis imperfecta (DI), blue sclerae, short stature, hearing loss, and cardiac malformations may be present (4). Variants in the collagen genes are responsible for approximately 85-90% of OI. Patients with OI have heterogeneous clinical and genetic features (5). Thus, clinical diagnosis is insufficient for optimal diagnosis, management, prognosis, and genetic counseling. In recent years, new OI types have been discovered with the development of improved genetic analysis techniques. The disorder can also be caused by variants of genes related to collagen structure and function (6,7). The most recently identified genes today are characterized by primary defects in osteoblast differentiation (2,3,6,8).

Advances in next-generation sequencing (NGS) technology have enabled the discovery of novel genes and pathogenic variants related to OI (2,9,10). Molecular diagnosis is useful for early diagnosis, prognosis, identifying other individuals in the family with the same variants, and deciding on optimal treatment based on the published evidence (2,9,10,11).

In this study, the aim was to investigate the molecular genetic etiology of OI using a targeted NGS panel and to determine the genotype-phenotype relationship in OI patients, and the effectiveness of this genetic panel for diagnosis.

Methods

Study Group

A cohort of clinically and/or radiologically diagnosed OI patients followed in Ege University Faculty of Medicine Pediatric Endocrinology and Diabetes Department were included in the study. Inclusion criteria were patients between 0-18 years of age with unknown molecular genetic etiology. Patients having any genetic disease other than OI that could cause bone fragility and other chronic diseases or patients with fragile bone syndrome due to medication, such as steroids or chemotherapy, were excluded.

Demographic data (age, gender, consanguinity, family history), clinical features (OI subgroup, frequency of annual bone fractures, treatment procedure and response), physical examination findings (bone deformities), and bone radiography findings were obtained from hospital records. Patients' weight and height and their standard deviation (SD) scores (SDS) were calculated based on Turkish standards (12,13).

The study was approved by the Ethics Committee of Ege University Faculty of Medicine (ethic committee number: 18-3.1/55, date: 20.03.2018), and samples from the patients were obtained in accordance with the Helsinki Declarations. Written informed consent for molecular analysis was obtained from all cases or their parents/guardians.

Molecular Analysis

Genomic DNA samples were extracted from leukocytes from 1 mL of peripheral blood obtained from all patients using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. DNA quality and quantity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For sequence analysis, a targeted NGS panel (TruSight One Panel by Illumina®) including 13 genes (*COL1A1, COL1A2, IFITM5, SERPINF1, CRTAP, P3H1, PPIB, SERPINH1, FKBP10, SP7, BMP1, MBTPS2, PLOD2)* associated with OI was used.

Data Analysis

Sequencing data was analyzed using Illumina VariantStudio software and IGV (Integrative Genomics Viewer). Firstly, 13 genes known to be responsible for OI were analyzed. Variants in these genes with a frequency of less than 0.5% in public databases including NCBI dbSNP build155 (http://www.ncbi.nlm.nih. gov/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), gnomAD (https://gnomad.broadinstitute.org/) and NHLBI Exome Sequencing Project Exome Variant Server (http://evs.gs.washington.edu/EVS/) were selected. The impact of the variants on the protein

structure was identified using several *in silico* prediction tools, such as MutationTaster, PolyPhen-2, and SIFT. The conservation of residues across species was evaluated by the PhyloP algorithm and GERP (14,15,16,17). The pathogenicity of all variants identified was classified according to the American College of Medical Genetics (ACMG) recommendations. The pathogenity scores were obtained from the https://www.acmg.net/ website. The ACMG Guidelines were established by clinicians and clinical lab directors who are experts in clinical genetics and members of the ACMG, the Association for Molecular Pathology (AMP), and/or the College of American Pathologists. Franklin Genoox software and database was used for ACMG Classification. There are 28 criteria in the ACMG guidelines. During variant interpretation, variants are classified into five tiers: pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign, and benign (B), depending on the applicable criteria. These criteria can be classified by the weight and type of evidence indicated by each criterion. The 28 criteria can be classified into eight types: population data, computational data, functional data, segregation data, *de novo* data, allelic data, other databases, and other data, depending on the source of evidence (18).

Confirmation

The most likely disease-causing variants, identified by data analysis, were confirmed using direct Sanger sequencing on ABI PRISM 3130 DNA analyzer (Applied Biosystems) and Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit (Life Technologies) (Fisher Scientific - Göteborg - Sweden).

Statistical Analysis

Analysis was conducted using Statistical Package for the Social Sciences for Windows, version 25.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics are reported as mean ± SD for normally distributed variables and median (range) for skewed data. Groups were compared using independent samples t-test for normally distributed variables and the Mann-Whitney U test for skewed data. Trends across time were analyzed using linear polynomial contrasts (ANOVA). A $p < 0.05$ was considered statistically significant. No adjustment was made for a multiplicity of statistical tests.

Results

Clinical Manifestations

Fifty-six patients (female/male: 25/31) from 46 different families were included in the study. In 15 (32.6%) families, consanguineous marriage was noted. The mean age of the cohort on admission was 4.5 ± 3.7 years, median body weight was -1.1 (-6.8, - 2.5) SDS, and height was -2.3 (-7.6, - 1.2) SDS. Based on the actualized Sillence classification (Table 1), 18 (33.1%) patients were considered to be type 1, 1 (1.7%) type 2, 26 (46.4%) type 3, and 11 (19.6%) type 4 (19). Bone deformity was detected in 30 (53.5%) of the patients, while 31 (55.4%) were evaluated as mobile. Thirty-six (60.7%) patients had blue sclera, 13 (23.2%) had scoliosis, 12 (21.4%) had DI, and 2 (3.6%) had hearing loss.

Molecular Analysis Findings

Sequence analysis of the *COL1A1* and *COL1A2* genes revealed heterozygous variants in 24 (52.1%) and 6 (13%) families, respectively. The (NM_000088.4(*COL1A1*):c.3677A > G/p.(Asp1226Gly)/rs1319157667) variant was detected together with the c.2296G > C variant in *COL1A2* in one patient. The variant detected in the *COL1A1* gene was also found in the asymptomatic father of the patient in segregation analysis. Based on this family history, she was excluded from the *COL1A1* group. The remaining 16 families were molecularly analyzed using the NGS panel, and in 8 (17.3%) families, a disease-causing variant in three different genes (*FKBP10, P3H1*, and *SERPINF*) was identified. Nine (23.6%) of the detected variants in all genes have not been previously reported and were considered to be deleterious based on prediction tools. Following a two-step NGS-based molecular analysis, a molecular diagnosis was achieved in 38 (82.6%) families in the study group.

Genotype-phenotype Relations

Fifteen of the *COL1A1* variants were boys, and 14 were girls. The mean age at admission was 4.69 ± 3.66 years, and weight and height SDS were -0.73 ± 1.39 and - 2.41 ± 4.45, respectively. The distribution of clinical diagnosis was as follows: 13 (44.8%) type 1; 1 (3.4%) type 2; 10 (34.4%) type 3; and 5 (17.2%) type 4. Recurrent pathological fractures

Table 1. The actualized Sillence classification (20)

OI type	
1	Mild form. Patients have no bone deformities, normal or near normal stature.
2	Extremely severe form is perinatal lethal.
3	Most severe form in children surviving the neonatal time, severely deforming, extreme short stature.
4	Intermediate form between type 1 and type 3: mild to moderate bone deformities and variable short stature.

OI: osteogenesis imperfecta

were detected in 25 (86.2%) of the patients, and deformity of extremities in 7 (24.1%) patients. Six (20.6%) patients were mobile with help or had the ability to sit. The rest of the patients were completely mobile. Twenty-five patients (86.2%) had blue sclera, and 8 (27.5%) had DI.

The mean age of admission of those with variants in the *COL1A2* gene (n = 6, 4 girls) was 5.17 ± 3.45 years, weight SDS was -2.86 ± 2.33, and height SDS was -3.07 ± 1.01. The distribution of clinical types was four (66.6%) type 3 and 2 (33.3%) type 4. In all patients, 2 or more recurrent pathological bone fractures and deformities were detected. In 5 (50%) patients, blue sclera and DI was found in one (16.6%) patient.

Biallelic variants in the *SERPINF1* gene were detected in four patients (patients 36, 37, 38, and 39) from three families. In one case, compound heterozygous variant c.80dupA/c.907C > T was present, and this patient's OI phenotype was compatible with type 3 with severe deformities, recurrent fractures, and short stature. However, family segregation was not performed in this patient.

A homozygous novel c.446T > G p.(Leu149Arg) variant was detected in the *P3H1* gene in a 0.2-year-old patient with a history of consanguineous marriage. The patient's weight SDS was -0.36, and height SDS was -2.12.

A homozygous c. 15dupC variant was identified in the *FKBP10* gene in a male patient with Bruck syndrome and clinically type 4 OI. Her parents were heterozygous for the same variant. The same variant was demonstrated in the sibling of the index case with a similar phenotype. These cases had severe OI and congenital contractures of large joints, short stature, and scoliosis.

Genotype and phenotype characteristics of patients with OI-related variants are given in Tables 2 and 3.

Discussion

The present study investigated the molecular etiology of 56 clinically diagnosed OI patients from 46 different families using an NGS panel, including a total of 4800 genes, including 13 genes related to OI. Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis with this TruSight One Panel. Such targeted gene panels are extremely reliable and validated and can be used in a wide range of indications for genetic diseases. Panels containing the genes of most diseases inherited as Mendelian in humans, such as the Illumina TruSight One Panel, are also referred to as "clinical exomes". This expression should not be confused with whole exome sequencing (WES) because approximately 20,000 genes detected in mankind are analyzed by WES analysis, while the clinical exome only contains genes associated with disease in humans. The advantage of the TruSight One panel compared to WES is that it is easier to analyze the results, and the cost is lower (20).

It is generally accepted that in-frame, partial deletions in the *COL1A1* or *COL1A2* genes can result in a lethal or severe OI phenotype when the resulting abnormal protein is not rapidly degraded but instead is incorporated in the triple helix exerting a dominant negative effect. In a study by (21), multiplex ligation-dependent probe amplification analysis was performed in the analysis of the *COL1A1* gene in a group of 106 index patients. These authors found seven patients with deletion of the complete *COL1A1* gene on one allele. In the present study, we did not evaluate the gross deletions and duplications in exons because this was outside the scope of the analysis methods. Although these types of variants are generally identified at low rates, they may be more relevant for the subgroup without any variants, in terms of disease severity through haploinsufficiency of *COL1A1* and *COL1A2* genes.

Consanguineous marriage was present in 28.5% of patients, and 39.3% had a family history of OI. Consanguineous marriage may lead to a high rate of autosomal recessive variants being found. In a study in India with seven patients of consanguineous marriage, *SERPINF1, PPIB*, and *CRTAB* mutations were detected (22). In a study evaluating *COL1A1* and *COL1A2* gene variants in 364 patients of Italian origin, the rate of positive family history rate was 57.7% (23). However, this increased rate of more than half may have been present because, with the exception of the two autosomal dominant gene variants in *COL1A1* and *COL1A2*, other types of OI were not studied. Family history rates were reported to be 53% in the Korean population (24), and 32-33% in different societies (25,26,27). The reason for these differences may be due to genetic differences in societies, variations in the genetic analysis methods used, and the changing frequency of *de novo* variants. Furthermore, the frequency of consanguineous marriage and/or founder variants in a society will lead to variability in the distribution of genes responsible for OI.

The clinical finding of blue sclera is one of the distinctive clinical features of OI and is frequently observed in patients with type 1 OI. Patients with type 3 and 4 OI may have blue sclera at birth, but the bluish color disappears with increasing age (2,8,28). In our study group, 34 (60.7%) of the patients had blue sclera.

Table 2. Genotype and phenotype characteristics of patients with variants related to osteogenesis imperfecta

Gene	Variant c.DNA (protein)	Consanguineous marriage in parents	Clinical type	Currently mobilization status	Number of fractures/ years	Bone deformity	BS	HL	DI	Patient number/ gender	Diagnosis age (yrs)
COL1A1	c.120C > A (p.Cys40*)	No	Type 3	Mobile	3	Yes, lower extremity	+	-	-	1/F	8.3
COL1A1	c.1283delG (p.Gly428Valfs*113)	No	Type 1	Mobile	3	No	+	-	-	2/F	1.8
			Type 1	Mobile	No	No	+	-	-	3/F	1.2
			Type 1	Mobile	3	No	+	-	-	4/M	6.7
COL1A1	c.1699C > T (p.Pro567Ser)	No	Type 4	Mobile	3	Yes, lower extremity	+	-	+	5/M	2.4
			Type 4	Mobile	No	No	+	-	+	6/M	6.1
COL1A1 / *COL1A2*	c.3677A > G (p.Asp1226Gly)/ c.2296G > C (p.Gly766Arg)	Yes	Type 4	Mobile	2	Yes, lower extremity	+	-	-	7/F	2.5 months
COL1A1	c.626G > A (p.Gly209Asp)	Unknown	Type 3	Assisted walking	3	Yes, very severe	+	-	-	8/M	8.5 months
COL1A1	c.1057-2A > C	Yes	Type 3	Mobile	6	No	+	-	-	9/M	3.7
COL1A1	c.1081C > T (p.Arg361*)	No	Type 1	Mobile	3	No	+	-	-	10/F	3.6
COL1A1	c.1299 + 1G > A	No	Type 1	Mobile	3	No	+	-	-	11/M	12.8
COL1A1	c.1299 + 1G > T	No	Type 1	Mobile	4	Yes, lower extremity	+	-	-	12/M	7.9
COL1A1	c.1353 + 2T > C	No	Type 3	Assisted walking	2	Yes, lower extremity	+	-	-	13/F	1.5 months
COL1A1	c.1405C > T (p.Arg469*)	No	Type 1	Mobile	2	No	+	-	+	14/F	3.4
COL1A1	c.2596G > A (p.Gly866Ser)	No	Type 2	Sitting	1	No	+	-	-	15/F	1.5 months
COL1A1	c.3235G > A (p.Gly1079Ser)	No	Type 3	Mobile	3	No	+	-	-	16/M	7.8
			Type 3	Mobile	3	No	+	-	-	17/M	10 months
COL1A1	c.3505G > A (p.Gly1169Ser)	No	Type 3	Assisted walking	3	Yes, lower extremity	+	-	-	18/F	10.6
COL1A1	c.1128delT (p.Gly377Alafs*164)	No	Type 1	Mobile	2	No	+	-	-	19/F	3.0
			Type 1	Mobile	3	No	+	-	-	20/M	2.9
COL1A1	c.1459_1460insA (p.Arg487Glnfs*6)	No	Type 1	Mobile	2	No	+	-	+	21/F	8.5 months
COL1A1	c.958G > C (p.Gly320Arg)	No	Type 4	Mobile	3	No	-	-	-	22/M	9.5
COL1A1	c.4051C > T (p.Gln1351*)	No	Type 3	Mobile	4	No	-	-	-	23/M	10.2
COL1A1	c.441delC (p.Gly148Aspfs*117)	No	Type 1	Mobile	3	No	+	-	+	24/M	7.1
COL1A1	c.886G > T (p.Gly296*)	No	Type 4	Mobile	2	No	+	-	-	25/F	3.9
COL1A1	c.1156-1G > A	Yes	Type 1	Mobile	1	No	+	-	-	26/F	4.6
COL1A1	c.3647A > G (p.Tyr1216Cys)	No	Type 4	Mobile	2	No	-	-	-	27/F	2.7
COL1A1	c.608G > T (p.Gly203Val)	No	Type 3	Sitting	5	Yes, lower extremity	+	-	+	28/M	1.7
COL1A1	c.1405C > T (p.Arg469*)	No	Type 1	Mobile	5	No	+	-	+	29/M	9.7
COL1A1	c.2829 + 2dupT	No	Type 3	Mobile	2	No	+	-	-	30/F	1.1
COL1A2	c.1972G > A (p.Gly658Ser)	No	Type 3	Assisted walking	4	Yes, lower extremity	-	-	-	31/F	10.3
COL1A2	c.3250G > T (p.Gly1084Cys)	No	Type 4	Assisted walking	2	Yes, lower extremity	+	-	-	32/M	10 months

Table 2. Continued

Gene	Variant c.DNA (protein)	Consanguineous marriage in parents	Clinical type	Currently mobilization status	Number of fractures/ years	Bone deformity	BS	HL	DI	Patient number/ gender	Diagnosis age (yrs)
COL1A2	c.928G > C (p.Gly310Arg)	Yes	Type 3	Assisted sitting	3	Yes, lower and upper extremity	+	-	+	33/M	3.5 months
COL1A2	c.1081G > A (p.Gly361Ser)	No	Type 3	Assisted walking	3	Yes, spine	-	-	-	34/F	5.7
COL1A2	c.3014G > A (p.Arg1005His)	No	Type 3	Immobile	3	Yes, very severe	-	+	-	35/F	6.5
SERPINF1	c.80dupA (p.Glu28Glyfs*37)/ c.907C > T (p.Arg303*)	No	Type 3	Immobile	4	Yes, very severe	-	-	-	36/F	8.5 months
SERPINF1	c.317G > C (p.Arg106Pro)	Yes	Type 1	Mobile	2	No	+	-	-	37/F	8.6
SERPINF1	c.988C > T (p.Gln330*)	Yes	Type 1	Mobile	2	No	+	-	-	38/M	11.6
			Type 3	Assisted sitting	4	Yes, spine	-	-	-	39/F	6 months
P3H1	c.446T > G (p.Leu149Arg)	Yes	Type 4	Mobile	4	Yes, lower extremity	-	-	-	40/F	2.5 months
P3H1	c.446T > G (p.Leu149Arg)	Yes	Type 3	Assisted walking	3	Yes, lower and upper extremity	-	-	-	41/F	1.5 months
FKBP10	c.1490G > A (p.Trp497*)	No	Type 4	Assisted walking	4	Yes, lower and upper extremity	-	-	-	42/M	4.3
			Type 4	Sitting	3	Yes, lower and upper extremity	-	-	-	43/M	5 months
FKBP10	c.831dupC (p.Gly278Argfs*95)	Yes	Type 3	Assisted sitting	3	Yes, lower and upper extremity	-	-	+	44/M	4.6
FKBP10	c.21dupC (p.Ser8Glnfs*67)	No	Type 3	Immobile	3	Yes, very severe	-	-	-	45/F	8.3
			Type 3	Assisted sitting	5	Yes, very severe	-	-	-	46/M	4.7
---	---	Yes	Sitting	Sitting	4	Yes, lower and upper extremity	+	-	-	47/F	6.2
---	---	Yes	Assisted sitting	Assisted sitting	3	Yes, very severe	-	-	-	51/M	3.7
---	---	Yes	Assisted sitting	Assisted sitting	3	Yes, very severe	+	-	-	53/M	1.5
---	---	No	Sitting	Sitting	3	Yes, lower extremity	-	-	-	48/M	13.2
---	---	No	Mobile	Mobile	1	No	+	-	-	49/M	10.6
---	---	No	Mobile	Mobile	3	Yes, upper extremity and spine	-	-	-	50/M	2.2
---	---	Yes	No sitting	No sitting	2	Yes, lower extremity	-	+	-	52/M	2.1
---	---	No	Mobile	Mobile	2	No	-	-	-	54/M	2.4
---	---	Yes	Mobile	Mobile	4	No	+	-	+	55/M	5.2
---	---	Yes	Assisted sitting	Assisted sitting	1	Yes, very severe	-	-	+	56/F	1.5 months

BD: bone deformity, BS: blue sclera, DI: dentinogenesis imperfecta, HL: hearing loss, M: male, F: female, yrs: years

Table 3. Genetic characteristics of patients with variants related to osteogenesis imperfecta

Gene	Variant c.DNA (protein)	Transcript	Genomic position	dbSNP	ACMG/AMP criteria	ExAC	GnomAD (aggregated)
COL1A1	c.120C > A (p.Cys40*)	NM_000088.4	chr17-48277292	rs762780039	P	N/A	N/A
COL1A1	c.1283delG (p.Gly428Valfs*113)	NM_000088.4	chr17-48272608		LP	N/A	N/A
COL1A1	c.1699C > T (p.Pro567Ser)	NM_000088.4	chr17-48271372		VUS	N/A	N/A
COL1A1	c.3677A > G (p.Asp1226Gly)/	NM_000088.4	chr17-48264138	rs1319157667	VUS	N/A	0.0032
COL1A2	c.2296G > C (p.Gly766Arg)	NM_000089.4	chr7-94050321		P		
COL1A1	c.626G > A (p.Gly209Asp)	NM_000088.4	chr17-48275326		P	N/A	N/A
COL1A1	c.1057-2A > C	NM_000088.4	chr17-48273028	rs66511271	LP	N/A	N/A
COL1A1	c.1081C > T (p.Arg361*)	NM_000088.4	chr17-48273002	rs72645366	P	N/A	N/A
COL1A1	c.1299 + 1G > A	NM_000088.4	chr17-48272592	rs66490707	P	N/A	N/A
COL1A1	c.1299 + 1G > T	NM_000088.4	chr17-48272592		LP	N/A	N/A
COL1A1	c.1353 + 2T > C	NM_000088.4	chr17-48272406	rs72648335	LP	N/A	N/A
COL1A1	c.1405C > T (p.Arg469*)	NM_000088.4	chr17-48272138	rs762428889	P	N/A	N/A
COL1A1	c.2596G > A (p.Gly866Ser)	NM_000088.4	chr17-48267237	rs67445413	P	N/A	N/A
COL1A1	c.3235G > A (p.Gly1079Ser)	NM_000088.4	chr17-48265483	rs72654802	P	N/A	N/A
COL1A1	c.3505G > A (p.Gly1169Ser)	NM_000088.4	chr17-48264402	rs67815019	P	N/A	N/A
COL1A1	c.1128delT (p.Gly377Alafs*164)	NM_000088.4	chr17-48272954	rs72645370	P	N/A	N/A
COL1A1	c.1459_1460insA (p.Arg487Glnfs*6)	NM_000088.4	chr17-48272083		LP	N/A	N/A
COL1A1	c.958G > C (p.Gly320Arg)	NM_000088.4	chr17-48273560		LP	N/A	N/A
COL1A1	c.4051C > T (p.Gln1351*)	NM_000088.4	chr17-48263336		P	N/A	N/A
COL1A1	c.441delC (p.Gly148Aspfs*117)	NM_000088.4	chr17-48276616	rs1473458290	P	N/A	N/A
COL1A1	c.886G > T (p.Gly296*)	NM_000088.4	chr17-48273862		LP	N/A	0
COL1A1	c.1156-1G > A	NM_000088.4	chr17-48272840		LP	N/A	N/A
COL1A1	c.3647A > G (p.Tyr1216Cys)	NM_000088.4	chr17-48264168	rs1555571849	LP	N/A	N/A
COL1A1	c.608G > T (p.Gly203Val)	NM_000088.4	chr17-48275344	rs72667031	P	N/A	N/A
COL1A1	c.1405C > T (p.Arg469*)	NM_000088.4	chr17-48272138	rs762428889	P	N/A	N/A
COL1A1	c.2829 + 2dupT	NM_000088.4	chr17-48266735		LP	N/A	N/A
COL1A2	c.1972G > A (p.Gly658Ser)	NM_000089.4	chr7-94047811		LP	N/A	N/A
COL1A2	c.3250G > T (p.Gly1084Cys)	NM_000089.4	chr7-94056590		P	N/A	N/A
COL1A2	c.928G > C (p.Gly310Arg)	NM_000089.4	chr7-94038912	rs72656391	LP	N/A	N/A

Table 3. Continued

Gene	Variant c.DNA (protein)	Transcript	Genomic position	dbSNP	ACMG/AMP criteria	ExAC	GnomAD (aggregated)
COL1A2	c.1081G > A (p.Gly361Ser)	NM_000089.4	chr7-94039599		LP	N/A	N/A
COL1A2	c.3014G > A (p.Arg1005His)	NM_000089.4	chr7-94055751	rs200357942	VUS	N/A	N/A
SERPINF1	c.80dupA (p.Glu28Glyfs*37)/ c.907C > T (p.Arg303*)	NM_002615.7	chr17-1670283		LP	0.0025	0.0046
			chr17-1679946	rs763291398	P		
SERPINF1	c.317G > C (p.Arg106Pro)	NM_002615.7	chr17-1674356	rs148872301	VUS	N/A	N/A
SERPINF1	c.988C > T (p.Gln330*)	NM_002615.7	chr17-1680027		LP	0.0016	0.0008
P3H1	c.446T > G (p.Leu149Arg)	NM_022356.4	chr1-43232197		VUS	0.0016	0.0008
FKBP10	c.1490G > A (p.Trp497*)	NM_021939.4	chr17-39977996		LP	N/A	N/A
FKBP10	c.831dupC (p.Gly278Argfs*95)	NM_021939.4	chr17-39975558	rs137853883	P	N/A	N/A
FKBP10	c.21dupC (p.Ser8Glnfs*67)	NM_021939.4	chr17-39969300	rs782271121	P	0.0189	0.0107

*Exome Aggregation Consortium (http://exac.broadinstitude.org).
#The allele frequency in the ExAC database does not represent all ethnic group.
LP: likely pathogenic, VUS: variant of unknown significance, P: pathogenic, N/A: not applicable, ACMG: American College of Medical Genetics, AMP: Association for Molecular Pathology

Bone fractures and deformities in OI usually occur at an early age and are often caused by repeated bone remodeling in long bones (2,29). This affects patients' growth, functional status, and mobility. In our study, 20.6% of patients with the *COL1A1* variant and all of those with the *COL1A2* variant had difficulty walking. In 24.1% of patients with the *COL1A1* variants, and all of those with the *COL1A2* variants, deformities were detected in the extremities. Mohd Nawawi et al., (30) showed that 63.6% of all OI patients had bone deformities at the age of nine years and needed help to walk. Studies have shown that bone deformities are more common in patients with qualitative variants than quantitative variants (2,29). Hald et al. (31,32) showed that OI patients with quantitative defects had normal protein structure in bone, despite collagen deficiency. This allows bone mineralization and thus leads to fewer breakages than qualitative defects (11,31).

DI has been reported in more frequently type 3 and rarely type 1 OI (33,34). DI was detected in 12 (21.4%) of the patients in the present study. The clinical diagnosis of the patients with DI was as follows: 5 patients (41.6%) were type 1, 4 (33.3%) type 3, and 3 (25%) type 4. In another study, DI was reported to be more frequent in patients with more severe clinical types (type 3 and 4) than in moderately affected groups (type 1) (34). Those with a qualitative variant, that is a problem in collagen structure, are more at risk of developing DI. Structurally abnormal collagen affects the development of dental germ cells in the predentin during the mineralization process (35).

In the present study, 63.1% had a variant in the *COL1A1* gene, 13.1% in the *COL1A2* gene, and 2.6% in both genes; in total, 78.8% of patients had variants in these two genes. In 11 (19.6%) out of 21 patients without variants in these genes, by NGS analysis, three other gene (*SERPINF1, FKBP10*, and *P3H1)* variants were detected. Three (7.8%) families had *FKBP10*, 3 (7.8%) families had *SERPINF1,* and 2 (5.2%) families had the *P3H1* variant. Abali et al. (36) studied 89 patients with OI. Similarly to our study, these authors reported a majority (61.4%) having variants in *COL1A1* and *COL1A2* genes, while much lower proportions had varinats in other OI-associated genes: 5 (5.6%) with *FKBP10*; 2 (2.2%) *LRP5*; 1 (1.1%) *P3H1*; 1 (1.1%) *CRTAP*; 1 (1.1%) *BMP1*; and 1 (1.1%) *SPARC* variant.

COL1A1 and *COL1A2* Gene Variants

Variants in *COL1A1* and *COL1A2* encoding type 1 collagen are responsible for most of the etiology in OI. In the present study, 62.4% of patients had variants in these two genes, similarly to previous repots where variants in these two genes were responsible for 51-73% of the disease (25,30,37,38).

Moreover, variants in the COL1A1 gene were detected more frequently in both the present study and earlier studies than the *COL1A2* gene (4,25,27,30).

In one (3.3%) case (patient 7), a heterozygous variant was detected in both *COL1A1* c.3677A>G p.(Asp1226Gly) and *COL1A2* c.2296G>C p.(Gly77Arg) genes. The variant detected in the *COL1A1* gene was also found in the asymptomatic father of the patient in segregation analysis and we made the decision to discount the VUS variant in the *COL1A1* gene as causing the phenotype. The variant in the *COL1A2* gene was previously reported as pathogenic. Therefore, this variant may be responsible for the clinical findings in this case, who had severe clinical type with recurrent fractures and severe deformities. No cases carrying variants in both these two genes simultaneously have been reported previously. However, Ji et al. (39) reported a case with a severe clinic variant in *COL1A1* and *SERPINF1*. Oligogenic inheritance should also be considered in cases with severe clinical features.

In patient 5, the variant c.1699C>T p.(Pro567Ser) in *COL1A1* gene. The variant c.1699C>T in COL1A1 was detected once in the GME Variome database and once in the Turkish Variome database. The frequency of the variant in GMA Variome was 0.05% and it was 0,02% in the Turkish Variome. Both frequencies are less than 0.06%, which is the cut-off level for ACMG-PM2 criteria for this gene, and this is supporting evidence for the possibly pathogenic nature of the variant. This variant has not been reported in association with OI or any disease.

P3H1 Gene Variants

A homozygous c.446T> G p.(Leu149Arg) variant was detected in the *P3H1* gene in two unrelated female patients (Patients 40 and 41) who were admitted with recurrent fractures before two months of age. This variant was thought to be disease-causing in *in silico* analysis and was not previously reported. Recurrent fractures continued with severe clinical phenotypes. In the literature, clinical OI type VIII due to *P3H1* gene variants have moderate/severe phenotypic features (31). In the West African community, in the *P3H1* gene, a relatively high c.1080+1G>T carriage was detected: 1/240. The homozygous form was associated with the perinatally lethal form of OI. This variant was thought to be a founding variant (40,41,42) but was not observed in any of the 56 patients in the present study.

SERPINF1 Gene Variants

In some populations, *SERPINF1* and *CRTAP* variants have been reported to be responsible for recessive OI types. Some variants have been suggested as causing a "founder" effect (43).

In the present study, four different variants were detected in three patients from different families, and another variant was detected in two siblings. Patients with *SERPINF1* variants had a more severe clinical picture and early admission. Compound heterozygous c.80dupA p.(Glu28Glyfs*37) / c.907C>T p.(Arg303Ter) variant was found in an infant (patient 36) with fractures from birth, widespread deformities, and severe short stature. In *in silico* analyses, both variants were disease-causing. No blue sclera, DI, or hearing loss was detected. In the follow-up, despite treatment, his fractures recurred, his deformities increased, and independent mobilization never developed. Another patient (patient 39) who presented with a severe clinical picture at the age of 6 months had a homozygous c.988C>T p.(Gln330Ter) variant. Vertebral and lower extremity fractures were present. Similar to these patients, most *SERPINF1* gene variants previously reported have been due to frameshift and nonsense variants (44,45). A missense homozygous c.317G>C p.(Arg106Pro) variant was found in a patient with a milder clinical picture who presented at 8.6 years with recurrent fractures without deformities. This variant was predicted to be a VUS by Franklin Genoox and Varsome programmes, by the ACMG 2015 criteria. Most of the predictions tool predicted that this variant will be pathogenic or VUS. This variant has low population frequency. Given this evidence, it was thought that this homozygous c.317G>C p.(Arg106Pro) variant detected in *SERPINF1* might be responsible for the clinical picture in the patient. These variants impair production of circulating pigment epithelium-derived factor (PEDF) as well as loss of PEDF protein function (2). Rauch et al. (46) reported that measuring PEDF concentration in serum may be a potential marker in assessing patients' clinical severity.

FKBP10 Gene Variants

A homozygous c.21dupC p.(Ser8Glnfs * 67) variant in the *FKBP10* gene was detected in an 8.3-year-old girl (patient 45) who presented with congenital joint contractures, recurrent fractures, and chest deformity resembling Bruck syndrome. No consanguineous marriage was reported. *FKBP10* gene variants have been associated with severe OI and Bruck syndrome (2,11). In two brothers with Bruck syndrome, Shaheen et al. (47) reported a homozygous 8-bp insertion variant in the *FKBP10* gene. Alanay and Krakow (48) reported that the patients in Shaheen et al.'s (47) study may have Bruck syndrome and that the clinical picture may be milder because they received bisphosphonate

treatment. Researchers have reported that different variants in the *FKBP10* gene can explain the variable severity of phenotypes.

Genotype-phenotype correlations in OI have been extensively studied over the years, with certain investigations revealing significant associations (49,50). Notably, more severe phenotypes have been observed in patients harboring pathogenic variants in *COL1A1* compared to those in *COL1A2* (51). Mrosk et al. (52) suggested a robust correlation between genotype and the severity of phenotypes. They proposed a ranking based on phenotype severity as follows: *P3H1, COL1A1*, and *COL1A2*, respectively. In the present study, 60% of the patients with variants detected in the *COL1A1* and *COL1A2* genes, 50% of the patients with variants detected in the *SERPINF1* and *P3H1* genes, and 60% of the patients with variants detected in the *FKBP10* gene had a severe phenotype. In addition, we identified a variant in a total of 15 affected individuals across seven families. Remarkably, the clinical types and features of cases with the same variant within these families were similar. On the other hand, c.1299+1G>A variant was detected in the *COL1A1* gene in cases 11 and 12 from different families, and c.446T>G variant was detected in the *P3H1* gene in cases 40 and 41. The clinical features of pateints 11 and 12 were similar. However, while case 41 was type 4 OI, case 41 was type 3 OI and case 41 showed more severe type features. The literature indicates that while varying phenotypes can exist within the same family, similarities can also exist among individuals from different families who share the same genetic variant (2,53). The genotype-phenotype relationship in OI remains variable, as carriers of the same variant may develop diverse phenotypes. Furthermore, the factors influencing additional phenotypic differences have yet to be fully elucidated.

In the present study, no variants were detected in any of the genes covered by TruSight One used in the targeted NGS analysis in 10 (17.8%) patients. There may be more OI-associated genes or perhpas new candidate genes that were not covered by this panel. Targeted gene panels are highly efficient in the diagnosis of genetic disorders, which have genetic heterogeneity. This panel gave an 82% diagnostic yield. For most of the cases this high diagnostic yield was clinically useful but current technology allows us to perform WES-CNV at a comparable price. However, in most centers the capacity of the genetic laboratories is the main determinant of the genetic approach.

In one study, it was noted that an unusually high percentage of AR forms due to mutations in genes such as *BMP1, FKBP10* were reported in their cohort of 50 patients. This highlights the utility of gene panel testing in a setting where specific mutations are known to be more common. WES can be particularly useful in cases where patients present with atypical features or where the targeted gene panel does not provide a definitive result. For example, in one patient, WES revealed no significant mutations, suggesting the presence of non-coding or complex inter-related genetic contributions to the disease that may have been missed by targeted panels. Targeted sequence analysis is often more practical and cost-effective when a patient's clinical presentation strongly points to mutations in known OI-associated genes. WES is more comprehensive and can reveal unexpected mutations, but is also more resource intensive. The choice between these techniques may depend on clinical indications, resources and the possibility of atypical genetic contribution to the disease (53).

Study Limitations

There are some limitations to the present study due to the small size of the study population, single-center nature, and possible selection bias due to being only one tertiary center. Furthermore, the cases' pedigrees were not considered because the study was conducted a long time ago. The exclusion of certain genes associated with osteogenesis imperfecta *(CCDC134, CREB3L1, KDELR2, MESD, SPARC, TENT5A, TNEN38B, WNT1)* also contributes to the study's limitations.

Conclusion

This fairly comprehensive study demonstrated the clinical and molecular features of OI in a clinically diagnosed Turkish cohort. Genetic etiology was identified in 82.6% of 46 families with targeted NGS analysis. In addition, nine novel variants in OI-associated genes were identified. However, no new candidate gene related to OI could be detected by NGS analysis and eight genes known to be associated with OI were not analyzed (see limitations above). In patients where variants cannot be detected by targeted NGS, advanced genetic analysis, such as WES analysis may be planned. Finally, targetted panel studies in genetically heterogeneous diseases like OI are helpful for increasing the rate of variant detection.

Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of the Ege University Medical Faculty (ethic committee number: 18-3.1/55, date: 20.03.2018), and samples from the patients were obtained in accordance with the Helsinki Declarations.

Informed Consent: Written informed consent for molecular analysis was obtained from all cases or their parents/guardians.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ferda Evin, Tahir Atik, Şükran Darcan, Özgür Çoğulu, Concept: Samim Özen, Design: Samim Özen, Damla Gökşen, Data Collection or Processing: Esra Işık, Hüseyin Onay, Bilçağ Akgün, Aysun Ata, Füsun Düzcan, Şükran Darcan, Özgür Çoğulu, Analysis or Interpretation: Hüseyin Onay, Bilçağ Akgün, Aysun Ata, Füsun Düzcan, Şükran Darcan, Özgür Çoğulu, Literature Search: Esra Işık, Hüseyin Onay, Tahir Atik, Ferda Özkınay, Writing: Damla Gökşen, Ferda Evin, Bilçağ Akgün, Aysun Ata, Füsun Düzcan.

Conflict of Interest: Two authors of this article, Samim Özen and Damla Gökşen, are a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, they were not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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References

1. Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. Nat Rev Endocrinol. 2011;7:540-557.
2. Rossi V, Lee B, Marom R. Osteogenesis imperfecta: advancements in genetics and treatment. Curr Opin Pediatr. 2019;31:708-715.
3. Palomo T, Vilaça T, Lazaretti-Castro M. Osteogenesis imperfecta: diagnosis and treatment. Curr Opin Endocrinol Diabetes Obes. 2017;24:381-388.
4. Erbaş İM, İlgün Gürel D, Manav Kabayeğit Z, Koç A, Ünüvar T, Abacı A, Böber E, Anık A. Clinical, genetic characteristics and treatment outcomes of children and adolescents with osteogenesis imperfecta: a two-center experience. Connect Tissue Res. 2022;63:349-358. Epub 2021 Jun 9
5. Gupta N, Gregory SW, Deyle DR, Tebben PJ. Three Patient Kindred with a Novel Phenotype of Osteogenesis Imperfecta due to a COL1A1 Variant. J Clin Res Pediatr Endocrinol. 2021;13:218-224. Epub 2020 Jun 10
6. Marini JC, Forlino A, Bächinger HP, Bishop NJ, Byers PH, Paepe A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. Nat Rev Dis Primers. 2017;3:17052.
7. Marini JC, Blissett AR. New genes in bone development: what's new in osteogenesis imperfecta. J Clin Endocrinol Metab. 2013;98:3095-3103. Epub 2013 Jun 14
8. Forlino A, Marini JC. Osteogenesis imperfecta review 2015, The Lancet 2015;363:1377-1385.
9. Patel R, Camacho P. Osteogenesis imperfecta: Case-Based Approach. Metab Bone Dis. 2019;123-135.
10. Morello R. Osteogenesis imperfecta and therapeutics. Matrix Biol. 2018;71-72:294-312. Epub 2018 Mar 11
11. Forlino A, Marini JC. Osteogenesis imperfecta. The Lancet. 2016;387:1657-1671.
12. Demir K, Konakçı E, Özkaya G, Kasap Demir B, Özen S, Aydın M, Darendeliler F. New Features for Child Metrics: Further Growth References and Blood Pressure Calculations. J Clin Res Pediatr Endocrinol. 2019;12:125-129. Epub 2019 Sep 2
13. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.
14. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013:7.
15. Goode DL, Cooper GM, Schmutz J, Dickson M, Gonzales E, Tsai M, Karra K, Davydov E, Batzoglou S, Myers RM, Sidow A. Evolutionary constraint facilitates interpretation of genetic variation in resequenced human genomes. Genome Res. 2010;20:301-310. Epub 2010 Jan 12
16. Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 2010;20:110-121. Epub 2009 Oct 26
17. J Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010;7:575-576.
18. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-423. Epub 2015 Mar 5
19. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014;164:1470-1481. Epub 2014 Apr 8
20. Loman NJ, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ. Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol. 2012;30:434-439.
21. van Dijk FS, Huizer M, Kariminejad A, Marcelis CL, Plomp AS, Terhal PA, Meijers-Heijboer H, Weiss MM, van Rijn RR, Cobben JM, Pals G. Complete COL1A1 allele deletions in osteogenesis imperfecta. Genet Med. 2010;12:736-741.
22. Stephen J, Girisha KM, Dalal A, Shukla A, Shah H, Srivastava P, Kornak U, Phadke SR. Mutations in patients with osteogenesis imperfecta from consanguineous Indian families. Eur J Med Genet. 2015;58:21-27. Epub 2014 Oct 24
23. Maioli M, Gnoli M, Boarini M, Tremosini M, Zambrano A, Pedrini E, Mordenti M, Corsini S, D'Eufemia P, Versacci P, Celli M, Sangiorgi L. Genotype-phenotype correlation study in 364 osteogenesis imperfecta Italian patients. Eur J Hum Genet. 2019;27:1090-1100. Epub 2019 Mar 18
24. Lee KS, Song HR, Cho TJ, Kim HJ, Lee TM, Jin HS, Park HY, Kang S, Jung SC, Koo SK. Mutational spectrum of type I collagen genes in Korean patients with osteogenesis imperfecta. Hum Mutat. 2006;27:599.
25. Lin HY, Chuang CK, Su YN, Chen MR, Chiu HC, Niu DM, Lin SP. Genotype and phenotype analysis of Taiwanese patients with osteogenesis imperfecta. Orphanet J Rare Dis. 2015;10:152.

26. Venturi G, Tedeschi E, Mottes M, Valli M, Camilot M, Viglio S, Antoniazzi F, Tatò L. Osteogenesis imperfecta: clinical, biochemical and molecular findings. Clin Genet. 2006;70:131-139.

27. Zhang ZL, Zhang H, Ke YH, Yue H, Xiao WJ, Yu JB, Gu JM, Hu WW, Wang C, He JW, Fu WZ. The identification of novel mutations in COL1A1, COL1A2, and LEPRE1 genes in Chinese patients with osteogenesis imperfecta. J Bone Miner Metab. 2012;30:69-77. Epub 2011 Jun 14

28. Bregou Bourgeois A, Aubry-Rozier B, Bonafé L, Laurent-Applegate L, Pioletti DP, Zambelli PY. Osteogenesis imperfecta: from diagnosis and multidisciplinary treatment to future perspectives. Swiss Med Wkly. 2016;146:w14322.

29. Tournis S, Dede AD. Osteogenesis imperfecta - A clinical update. Metabolism. 2018;80:27-37.

30. Mohd Nawawi N, Selveindran NM, Rasat R, Chow YP, Abdul Latiff Z, Syed Zakaria SZ, Jamal R, Abdul Murad NA, Abd Aziz BB. Genotype-phenotype correlation among Malaysian patients with osteogenesis imperfecta. Clin Chim Acta. 2018;484:141-147. Epub 2018 May 25

31. Hald JD, Folkestad L, Harsløf T, Lund AM, Duno M, Jensen JB, Neghabat S, Brixen K, Langdahl B. Skeletal phenotypes in adult patients with osteogenesis imperfecta-correlations with COL1A1/COL1A2 genotype and collagen structure. Osteoporos Int. 2016;27:3331-3341. Epub 2016 Jun 2

32. Saeves R, Lande Wekre L, Ambjørnsen E, Axelsson S, Nordgarden H, Storhaug K. Oral findings in adults with osteogenesis imperfecta. Spec Care Dentist. 2009;29:102-108.

33. Andersson K, Dahllöf G, Lindahl K, Kindmark A, Grigelioniene G, Åström E, Malmgren B. Mutations in COL1A1 and COL1A2 and dental aberrations in children and adolescents with osteogenesis imperfecta - A retrospective cohort study. PLoS One. 2017;12:e0176466.

34. Lindahl K, Åström E, Rubin CJ, Grigelioniene G, Malmgren B, Ljunggren Ö, Kindmark A. Genetic epidemiology, prevalence, and genotype-phenotype correlations in the Swedish population with osteogenesis imperfecta. Eur J Hum Genet. 2015;23:1042-1050. Epub 2015 May 6

35. Malmgren B, Andersson K, Lindahl K, Kindmark A, Grigelioniene G, Zachariadis V, Dahllöf G, Åström E. Tooth agenesis in osteogenesis imperfecta related to mutations in the collagen type I genes. Oral Dis. 2017;23:42-49. Epub 2016 Sep 13

36. Abali S, Arman A, Atay Z, Bereket A, Bas S, Haliloglu B, Guran T, Gormez Z, Demirci H, Akarsu N, Turan S. Frequency of Recessive Osteogenesis Imperfecta in a Turkish Cohort and Genetic Causes. 55th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE), Paris, France, September 10-12, 2016: Abstracts, Horm Res Paediatr. 2016;86(Suppl 1):175-176.

37. Ho Duy B, Zhytnik L, Maasalu K, Kändla I, Prans E, Reimann E, Märtson A, Kõks S. Mutation analysis of the COL1A1 and COL1A2 genes in Vietnamese patients with osteogenesis imperfecta. Hum Genomics. 2016;0:27.

38. Patel RM, Nagamani SC, Cuthbertson D, Campeau PM, Krischer JP, Shapiro JR, Steiner RD, Smith PA, Bober MB, Byers PH, Pepin M, Durigova M, Glorieux FH, Rauch F, Lee BH, Hart T, Sutton VR. A cross-sectional multicenter study of osteogenesis imperfecta in North America - results from the linked clinical research centers. Clin Genet. 2015;87:133-140. Epub 2014 May 30

39. Ji Y, Ikram A, Ma Z, Peppelenbosch MP, Pan Q. Co-occurrence of heterozygous mutations in COL1A1 and SERPINF1 in a high-risk pregnancy complicated by osteogenesis imperfecta. J Genet. 2019;98:51.

40. Cabral WA, Barnes AM, Adeyemo A, Cushing K, Chitayat D, Porter FD, Panny SR, Gulamali-Majid F, Tishkoff SA, Rebbeck TR, Gueye SM, Bailey-Wilson JE, Brody LC, Rotimi CN, Marini JC. A founder mutation in LEPRE1 carried by 1.5% of West Africans and 0.4% of African Americans causes lethal recessive osteogenesis imperfecta. Genet Med. 2012;14:543-551. Epub 2012 Jan 26

41. Baldridge D, Schwarze U, Morello R, Lennington J, Bertin TK, Pace JM, Pepin MG, Weis M, Eyre DR, Walsh J, Lambert D, Green A, Robinson H, Michelson M, Houge G, Lindman C, Martin J, Ward J, Lemyre E, Mitchell JJ, Krakow D, Rimoin DL, Cohn DH, Byers PH, Lee B. CRTAP and LEPRE1 mutations in recessive osteogenesis imperfecta. Hum Mutat. 2008;29:1435-1442.

42. Pepin MG, Schwarze U, Singh V, Romana M, Jones-Lecointe A, Byers PH. Allelic background of LEPRE1 mutations that cause recessive forms of osteogenesis imperfecta in different populations. Mol Genet Genomic Med. 2013;1:194-205. Epub 2013 Jun 26

43. Bardai G, Moffatt P, Glorieux FH, Rauch F. DNA sequence analysis in 598 individuals with a clinical diagnosis of osteogenesis imperfecta: diagnostic yield and mutation spectrum. Osteoporos Int. 2016;27:3607-3613. Epub 2016 Aug 11

44. Al-Jallad H, Palomo T, Roughley P, Glorieux FH, McKee MD, Moffatt P, Rauch F. The effect of SERPINF1 in-frame mutations in osteogenesis imperfecta type VI. Bone. 2015;76:115-120. Epub 2015 Apr 11

45. Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C. Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. Am J Hum Genet. 2011;88:362-371. Epub 2011 Feb 25

46. Rauch F, Husseini A, Roughley P, Glorieux FH, Moffatt P. Lack of circulating pigment epithelium-derived factor is a marker of osteogenesis imperfecta type VI. J Clin Endocrinol Metab. 2012;97:1550-1556. Epub 2012 Jun 5

47. Shaheen R, Al-Owain M, Sakati N, Alzayed ZS, Alkuraya FS. FKBP10 and Bruck syndrome: phenotypic heterogeneity or call for reclassification? Am J Hum Genet. 2010;87:306-307.

48. Alanay Y, Krakow D. Response to Shaheen et al. Am J Hum Genet. 2010;87:306-308.

49. Rauch F, Lalic L, Roughley P, Glorieux FH. Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. J Bone Miner Res. 2010;25:1367-1374.

50. Ben Amor IM, Glorieux FH, Rauch F. Genotype-phenotype correlations in autosomal dominant osteogenesis imperfecta. J Osteoporos. 2011;2011:540178. Epub 2011 Sep 6

51. Zhytnik L, Maasalu K, Pashenko A, Khmyzov S, Reimann E, Prans E, Kõks S, Märtson A. COL1A1/2 Pathogenic Variants and Phenotype Characteristics in Ukrainian Osteogenesis Imperfecta Patients. Front Genet. 2019;10:722.

52. Mrosk J, Bhavani GS, Shah H, Hecht J, Krüger U, Shukla A, Kornak U, Girisha KM. Diagnostic strategies and genotype-phenotype correlation in a large Indian cohort of osteogenesis imperfecta. Bone. 2018;110:368-377. Epub 2018 Feb 27

53. Lim J, Grafe I, Alexander S, Lee B. Genetic causes and mechanisms of Osteogenesis Imperfecta. Bone. 2017;102:40-49. Epub 2017 Feb 15

ORIGINAL ARTICLE

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Treatment of Severe Hyperglycemia in Extremely Preterm Infants Using Continuous Subcutaneous Insulin Therapy

Merle Böettger[1], Tony Zhou[2], Jennifer Knopp[2], J. Geoffrey Chase[2], Axel Heep[1], Michael von Vangerow[1], Eva Cloppenburg[1], Matthias Lange[1]

[1]Carl von Ossietzky University of Oldenburg, School of Medicine and Health Sciences, Perinatal Neurobiology Research Group, Oldenburg, Germany
[2]University of Canterbury, Department of Mechanical Engineering, Christchurch, New Zealand

What is already known on this topic?

Hyperglycemia is common in preterm infants and can affect long-term outcomes, especially regarding the neurological outcome. However, there are no treatment guidelines and there are a lot of controversies. Insulin is mostly administered via continuous intravenous infusion in preterm infants. Continuous subcutaneous insulin infusion (CSII) in preterm infants is only described in a few case reports and clinical research papers.

What this study adds?

This study adds clinical expertise in the application of CSII in extremely preterm infants. The comparison of CSII and intravenously given Insulin in preterm infants allows to draw conclusions on the feasibility and dose control of CSII in preterm infants. To our knowledge it is the largest, described cohort of extremely preterm infants receiving CSII.

Abstract

Objective: Hyperglycemia in preterm infants is usually treated with adjustment of glucose intake and, if persistent, with continuous insulin infusion. However, hypoglycemia is a well-known complication of intravenous (iv) insulin treatment. The aim of this study was to evaluate the feasibility of continuous subcutaneous insulin infusion (CSII) in extremely preterm infants.

Methods: Clinical data from extremely premature infants (< 28 weeks of gestation) undergoing CSII treatment for severe hyperglycemia in the neonatal intensive care unit were included. Blood glucose levels during CSII, as well as the nutritional intake and insulin intake were recorded. Data were analyzed and compared to a control group of very preterm infants receiving iv insulin therapy.

Results: Normoglycemia rates were best in the iv insulin-cohort (n = 22, 50.3 %) compared to the CSII group (n = 15, 15.6 %). Hypoglycemia was very rare in both groups (0.4 % vs. 0.0 %). CSII therapy appears to require higher insulin doses compared to continuous iv therapy to achieve a similar effect. Subcutaneous Insulin therapy in extremely preterm infants is feasible, at least for prevention of hypoglycemia. However, dose control needs to be improved.

Conclusion: The results justify further model validation and clinical trial research to explore a model-based protocol and the use of CSII in this population.

Keywords: Continuous subcutaneous insulin infusion, extremely preterm infants, hyperglycemia

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Address for Correspondence: Merle Böttger MD, Carl von Ossietzky University of Oldenburg, School of Medicine and Health Sciences, Perinatal Neurobiology Research Group, Oldenburg, Germany
E-mail: merle.hannah.boettger@uol.de **ORCID:** orcid.org/0009-0004-8969-3532

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Introduction

In terms of long-term neurocognitive development, prevention of hypo- and hyper-glycemia plays a major role in the care of premature infants (PI). Persistent hyperglycemia occurs mostly in very low birth weight infants (VLBW) in the first days to weeks of life (1,2). There is a negative correlation between gestational age (GA), birth weight, and the occurrence of hyperglycemic episodes (1). In this respect, an isolated blood glucose (BG) level >10 mmol/L within the first 28 days of life in VLBW is associated with a more than two-fold increase in 28-day mortality (3). Furthermore, hyperglycemia within the first 24 hours of life is associated with reduction in brain white matter structure on magnetic resonance imaging (4).

Thresholds for managing hyperglycemia vary considerably across clinical settings (5,6,7,8,9). Due to varying definitions and methodological differences for BG level assessment, the incidence of hyperglycemia in studies varies between 40-80 % (1,3,10,11). The prevalence of hyperglycemia is highest at the end of the second week of life with approximately 30 % of preterm infants below 1500 g presenting with BG levels >10 mmol/L (10,11). Management of hyperglycemia starts with the adjustment of glucose intake. with the clinical aim of a reduction to a basic requirement of 5-6 g/kg/day. Insulin treatment has been introduced using continuous intravenous (iv) infusion (3,12,13,14,15). However, increased catheter-associated infections have been described with iv treatment (2). In addition, the use of iv insulin infusion involves the fear of a resulting, iatrogenic hypoglycemia. Although continuous subcutaneous insulin infusion (CSII) therapy is regarded as a standard treatment of diabetes management in the pediatric population, very few data exist for using CSII in neonatal hyperglycemia. Available data are limited to a few studies in connection with neonatal diabetes mellitus (16,17), case reports, or "close loop" monitoring in neonates (6,7,8). Hence, there is a gap in knowledge, which might offer benefit.

In the neonatal intensive care unit (NICU) of the University of Oldenburg, CSII in the management of hyperglycemia of extremely preterm infants was introduced as part of standard care since 2015. The aim of this study was to review the management of hyperglycemia following a standard CSII protocol in view of feasibility and safety. Data are compared with a cohort of preterm infants, treated for hyperglycemia within the first weeks of life using iv insulin from the NICU in Christchurch Hospital, New Zealand.

Methods

In a retrospective multi-center observational study 15 extremely preterm infant, receiving CSII treatment for severe hyperglycemia in the first weeks of life during the period 01/01/2015 to 01/04/2021, were identified. All infants were inborn patients treated at the level 3 NICU of the University of Oldenburg. Data on patient characteristics, BG test results, insulin medication, enteral nutritional intake, administration of parenteral iv infusion and individual drug medication were collected from patient records. Enteral glucose was supplied either as breast milk or preterm formula. For breast milk, a carbohydrate content of 70 mg per liter was estimated. The carbohydrate content of preterm formula was calculated according to the manufacturer's instructions.

All BG measurements were performed by rapid Accu-Chek BG test (Roche Diabetes Care Inc., Indianapolis, Indiana, USA). The definition of hyper- and hypo-glycemia, as well as the therapeutic intervention in the management of transient hyperglycemia, followed the local NICU guideline: Hypoglycemia, <4 mmol/L; life-threatening hypoglycemia, <2.6 mmol/L; normoglycemia, 4-10 mmol/L; and hyperglycemia, >10 mmol/L. For BG values >16.65 mmol/L repeat measurement was performed within 12 hours, reduction of parenteral glucose infusion rate in 1-1.5 g/kg/day steps to a minimum of 5-6 mg/kg/hour was performed initially. The indication for continuous insulin therapy was when BG values >16.65 mmol/L persisted over a period of 12 hours, despite adjustment of the iv glucose rate to a minimum of 5-6 g/kg/d. According to our guideline, the initial dosing of CSII was 0.01-0.05 IU/kg/hour, increased in small increments to a maximum rate of 0.1 IU/kg/hour, depending on measured BG values. Insulin dosing was re-evaluated and modified at the bedside with each 3-hour BG measurement. A BG level between 8.3-11.2 mmol/L (150-200 mg/dL) was the clinical goal for the BG range during CSII.

All preterm infants used an Accu-Chek Combo insulin pump (Roche Diabetes Care Inc., Indianapolis, Indiana, USA) with rapid-acting (2-5 hour action duration) Humalog insulin (Eli Lilly Co., Indianapolis, Indiana, USA). The insulin pumps were not designed for use in preterm infants, so the standard insulin concentration of 100 IU/mL would have limited delivery in PI. Therefore, the insulin was diluted 1/10 with "Sterile Diluent for Humalog U-100" to achieve a concentration of 10 IU/mL. The subcutaneous needle was placed in the thigh of the patients and was routinely changed every 48-72 hours, following guidelines. The insulin in the pump was routinely changed every seven days.

Results were compared with a retrospective, iv insulin-treated cohort of preterm infants, consisting of 22 VPI at the NICU in Christchurch, New Zealand between 2005-2009. Analogous to the procedure in Oldenburg, a reduction in glucose intake was carried out in the corresponding study period in the case of persistent hyperglycemia. With two values above 10 mmol/L, intravenous insulin therapy with 0.05 IU/kg/hour was started. In the course, a fixed adjustment was made depending on the BG value.

Ethics approval to conduct this study was obtained from the Medical Ethics Committee of the University of Oldenburg (no: 2021-024, date: 11.02.2021).

Statistical Analysis

For statistical analysis, descriptive tests were used. The distribution of values was non-Gaussian. Statistical analyses were processed using Statistical Package for the Social Sciences statistical software, version 26.0 for Mac (IBM Inc., Armonk, NY, USA).

Results

Patient characteristics are presented in Table 1. The SCII group (n = 15) of VPI presented with a median birth-weight of 620 [interquartile range (IQR): 560-700] g and GA of 24 (24 + 0 - 24 + 6) weeks. CSII was initiated at a median of 77 (61-141) hours of life. The median duration of insulin therapy using CSII was 191 (71-244) hours in the cohort. A total of 2.736 hours of insulin therapy by CSII and 803 glucose readings were included. No major complications requiring treatment occurred. Local redness at needle insertion site was observed twice, and both healed spontaneously.

The iv-cohort was composed of 22 preterm infants (7 male, 31.8%) who received continuous iv insulin in the NICU at the Christchurch Women's Hospital. Median GA was 27 (26-27) weeks with a median birth weight of 840 (800-900) g. Median duration of therapy was 86 (32.5-184) hours. Table 1 depicts patient and therapeutic characteristics of both cohorts.

Table 1 lists key delivery and outcome glycemia results for both cohorts. For the Oldenburg CSII cohort, the starting dose used was a minimum of 0.002 IU and a maximum of 0.102 IU/h/kg, with a median of 0.014 (0.036-0.010) IU/h/kg. The minimum insulin intake in this cohort ranged from 0.002 to 0.051 IU/h/kg, spanning 0.049 IU/h/kg body weight. The maximum insulin dose ranged from 0.011 to 0.181 IU/h/kg. A total of 40% (6/15 VPI) received insulin doses above the maximum recommended dose of 0.1 IU/h/kg (Figure 1), where all infants had highly variable administration rates.

Table 1. Cohorts and demographic data for the CSII (Oldenburg, Germany) cohort and the iv insulin treated cohort (Christchurch, New Zealand). Data are shown as median value and (range) or (IQR) as shown

	CSII cohort (Oldenburg)	iv cohort (Christchurch)
n patients (n male)	15 (8)	22 (7)
Total hours of treatment	2736	2946
Amount of BG measurements	803	902
Duration of insulin application, hours [IQR]	191 [71-249]	86 [32.5-184]
Gestational age*, weeks [IQR]	24 [24-24]	27 [26-27]
Body weight*, gram [IQR]	620 [570-735]	840 [800-900]
Postnatal age, days [IQR]**	3 [2-5]	3 [1-7]
BG measurement interval, hours [IQR]	3.3 [2.9-3.9]	3.4 [2.8-3.9]
Insulin rates, IU/kg/h [IQR]	0.02 [0.02-0.05]	0.03 [0.02-0.05]
Total glucose input, mg/kg/min [IQR]	8.0 [6.8-9.6]	8.5 [5.5-9.2]
BG median [IQR]	11.2 [9.9-12.5]	7.9 [6.6-9.2]
Median % BG between 4.0-8.0 mmol/L [IQR] (mean)	15.6 [5.6-21.1] (13.2)	50.3 [42.1-66.3] (50.9)
Median % BG > 10 mmol/L [IQR] (mean)	62.8 [50.0-77.5] (65.0)	8.1 [4.9-21.4] (17.2)
Median % BG < 4.0 mmol/L [IQR] (mean)	0.0 [0.0-1.3] (0.5)	0.4 [0.0-3.0] (2.1)
Median % BG < 2.6 mmol/L [IQR] (mean)	0.0 [0.0-0.0] (0.1)	0.0 [0.0-0.0] (0.1)
Number of patients with BG level < 2.6 mmol/L	1	1

*At birth.
**At the start of insulin treatment.
IQR: interquartile range, CSII: continuous subcutaneous insulin infusion, iv: intravenous, BG: blood glucose

The interquartile range of insulin intake of the 15 VPI of 0.02-0.05 IU/kg/h illustrates a high variability of the insulin dose used around the median of 0.02 IU/h/kg. In 3 (20%) the starting dose was below the recommended minimum of 0.01 IU/h/kg at baseline. In contrast, 6 (40%) VPI received an insulin supply above the recommended maximum of 0.1 IU/h/kg. The median insulin intake for the entire duration of therapy was below the possible recommended maximum starting dose of 0.05 IU/h/kg in 11/15 (73.3%) preterm infants.

The 22 patients in the comparison group receiving iv insulin as a continuous insulin infusion had a median insulin dose of 0.03 IU/h/kg (Table 1). Insulin delivery rates had a higher median value, but similar range and IQR. Glucose administration was similar between the two cohorts. The evaluation of BG using CSII was based on the percentage of measured values in a defined range for normo-, hypo- and hyper-glycemia. Only values measured during CSII insulin administration (>0.0 IU/kg/h) were analyzed. The percentages refer to the number of glucose readings of the individual n=15 patients. Figure 2 shows the percentages of individual patients results. In 10/15 (66.7%) infants of the CSII cohort, contrary to expectations, the greater proportion of BG readings (58.8-100%) were above the defined reference range. In 5 (33%) of this cohort, 50% to a maximum of 55% of the BG readings were within the reference range. The maximum proportion of normoglycemia for each

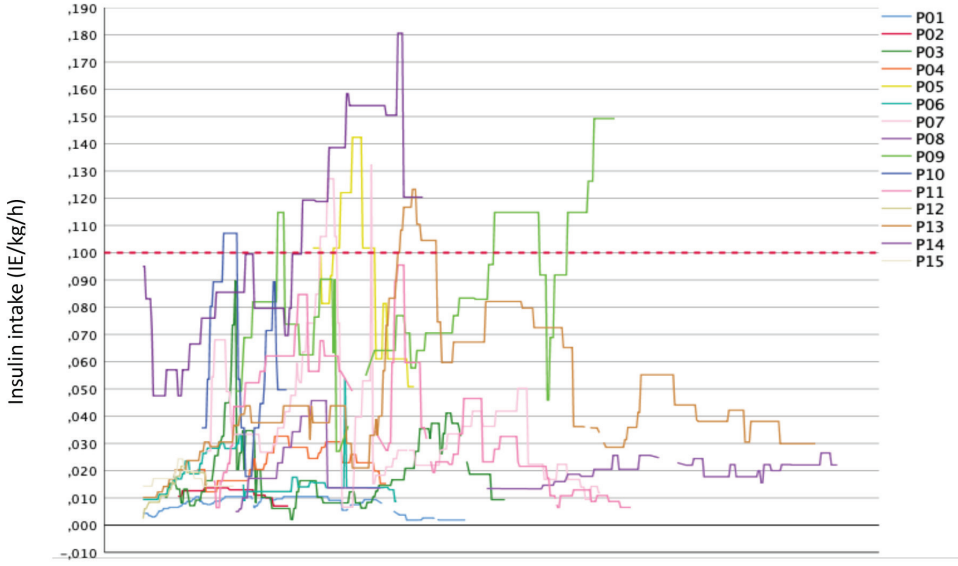

Figure 1. Insulin intake of VPI (n=15) suffering from persistent hyperglycemia

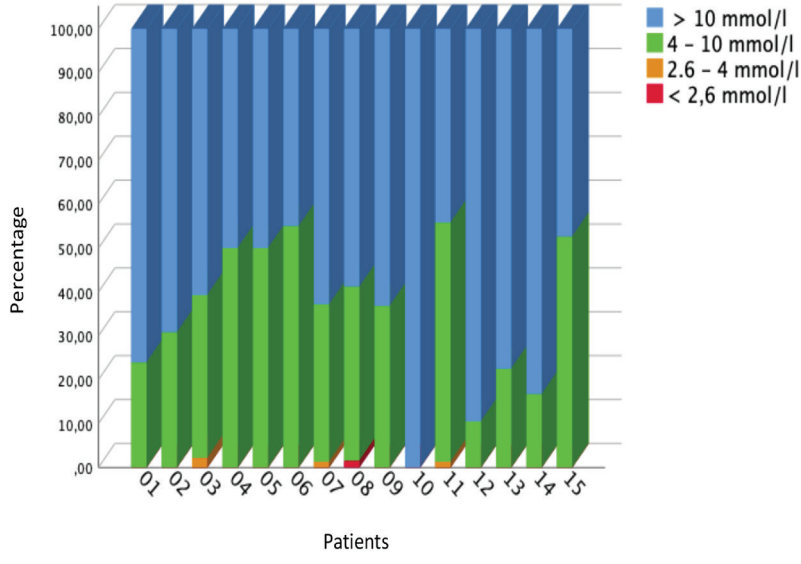

Figure 2. Percentage of glucose measured in plasma for each of the n=15 preterm infants

preterm infant considered individually was not very high, at 55%. This outcome may be clarified when considering all 803 glucose readings. Overall, across the CSII cohort, only 34.5% were between 4.0-10.0 mmol/L and only 13.2% between 4.0-8.0 mmol/L. Results and time in the normoglycemic range were relatively much higher for the iv insulin treated cohort (Table 1).

Discussion

Severe hyperglycemia in extremely preterm infants is associated with numerous comorbidities, which may persist into adulthood (4,8,18,19). This highlights the need for adequate therapy, which should compensate for the metabolic instability and/or functional insufficiency of compensating mechanisms during the first weeks of life of extremely preterm infants (1,2,3,11). The causes of dysregulation of glucose metabolism in preterm infants are diverse and include inadequate insulin production, low glycogen stores, and possible insulin resistance (7). In addition, treatment of hyperglycemia with continuous iv insulin is difficult and requires close monitoring to avoid associated hypoglycemia. Hyperglycemia has been shown as a first sign of cerebral intraventricular hemorrhage and at the same time being attributed to its development (20). As early as 1986, Ostertag et al. (21) provided evidence that extremely preterm infants may benefit from insulin pump therapy. Previous findings provide evidence for lower glucose fluctuations due to insulin pump therapy with continuous glucose measurement, compared to continuous intravenous insulin administration (6,8).

In the present study, 15 extremely preterm infants who received insulin subcutaneously via an insulin pump were studied. During the therapy period of more than 2.700 hours, no local or systemic infections requiring treatment were observed. Furthermore, only one episode of severe hypoglycemia was observed in more than 800 BG measurements. Thus, the therapeutic use of subcutaneous insulin therapy in VPI can be considered safe. However, in this study no CGM was used and thus hypoglycemia might have gone undetected.

The preterm infants in the CSII cohort and a comparator iv insulin cohort received similar insulin administration rates (CSII: 0.02 IU/h/kg; iv insulin: 0.03 IU/h/kg). However, the iv insulin cohort had a significantly higher proportion of normoglycemic readings. Thus, the glycemic control of the CSII cohort appears inadequate at similar insulin rates. However, the birth weight, as well as the GA of the CSII cohort was lower than the iv cohort. The results suggest the different kinetics of iv insulin versus subcutaneous insulin therapy, and particularly the potential for subcutaneous insulin losses, which may explain the differences. Hence, the results suggest CSII in these cohorts may require a higher insulin dose, especially at start of the treatment, compared to iv insulin.

In the cohort studied, the median starting CSII insulin dose was within the in-house recommended range of 0.01-0.05 IU/h/kg. However, there was marked variability. The median value also corresponds to the dosage of insulin used in previous studies (9,22). Compared with the cohort of continuous intravenous insulin delivery, the insulin rate of the CSII cohort was lower, even though possible insulin losses may reduce its impact. This difference may also be attributed to differences in protocol between the units and a different level of acceptance regarding safe insulin dosing levels. The “hesitant” use of insulin contrasts with the high BG values before the start of therapy and the high proportion of hyperglycemia during therapy. The reason for this may be the risk associated with hypoglycemia and a desire to avoid this, which is certainly of high priority for preterm infants. However, to achieve continuous normoglycemia, adequate insulin dosing is essential. Moreover, persistent hyperglycemia (>180 mg/dL or 10 mmol/L) is also associated with worse outcome in preterm infants (13,23). However, the lack of treatment recommendations when using CSII makes adequate glycemic control difficult. Avoiding hyperglycemia by means of adequate insulin delivery should be seen as important as avoiding hypoglycemia.

In the 15 preterm infants studied, adequate glycemic control could not be achieved using a CSII with the insulin rates used. Overall, this study is a comparison of cohorts with differences in sample number, GA, and birth weight. Nevertheless, descriptive comparisons can be made because the number of glucose measurements and the total duration of insulin administration are similar. The importance of adequate insulin dosing is evident when considering the high proportion of hyperglycemia in the cohort studied. Severe hyperglycemia is associated with worse outcome in preterm infants (23). For example, Kao et al. (23) demonstrated a significant association between hyperglycemia (mean 7-day glucose >180 mg/dL or 10 mmol/L) and the occurrence of necrotizing enterocolitis II°-III°. In addition, hyperglycemia >8 mmol/L in extremely preterm infants appears to be associated with delayed motor development and lower intelligence quotients at 6.5 years of age. Insulin therapy, on the other hand, appears to have no effect on either outcome (24). Current data suggest model-based insulin administration has the potential to improve therapy management. STAR-GRYPHON is a metabolic model that already improves the control of continuous iv insulin

therapy, considering factors such as enteral and parenteral glucose intake, weight and age. In the NICU in Christchurch, New Zealand, it has been used in clinical practice for some time (25). In a recent study, Zhou et al. (26) demonstrated model-based subcutaneous insulin therapy may allow for better control to achieve the goal of normoglycemia more rapidly and persistently.

Study Limitations

The small number of cases in the study limits the conclusions that can be drawn from this analysis. Furthermore, the iv insulin cohort comparator is not randomized nor matched and was born around ten years before the CSII Group. In the last ten years there have been many changes in the practice of neonatology, which might also affect the outcome. The CSII cohort received insulin in a neonatal center with different in-hospital standards and protocols. The iv treatment protocol had, for example, a lower treatment threshold, which has to be considered when looking at the results. In addition, the preterm infants in the iv insulin treated cohort had a higher median GA and birth weight and a higher case load. Due to the small number of cases the calculation of p values to compare continuous non-parametric groups of values was waived. However, the differences and similarities in glycemic outcomes allow conclusions to be drawn on the safety and potential efficacy of CSII in these cohorts and the need to better account for differences in insulin kinetics between delivery routes.

Conclusion

Overall, the comparison of the two cohorts allows an indication of inadequate glycemic control and insulin rates in the CSII cohort. This study shows CSII in extremely preterm infants is feasible but, compared with the retrospective iv insulin treated cohort, the current insulin regime leads to insufficient control of hyperglycemia. In terms of hypoglycemia, as well as local infections, CSII in extremely preterm infants appears quite safe. However, in view of different kinetics compared to iv therapy, there is still considerable potential for improvement in dosing. CSII required higher dosing of insulin compared to iv administered insulin. Ideally, the mode of administration should be model-based to best account for inter- and intra-patient variability in kinetics and dynamics of insulin action. Randomized studies with an adequate number of cases are necessary once safe, effective treatment protocols have been established.

Ethics

Ethics Committee Approval: Ethics approval to conduct this study was obtained from the Medical Ethics Committee of the University of Oldenburg (no: 2021-024, date: 11.02.2021).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: Axel Heep, Design: Axel Heep, Matthias Lange, Data Collection or Processing: Merle Böettger, Tony Zhou, Analysis or Interpretation: Merle Böettger, Jennifer Knopp, J. Geoffrey Chase, Axel Heep, Michael von Vangerow, Eva Cloppenburg, Matthias Lange, Literature Search: Merle Böettger, Writing: Merle Böettger, J. Geoffrey Chase, Axel Heep, Matthias Lange.

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References

1. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, Vanhole C, Palmer CR, Ong K, vanWeissenbruch M, Midgley P, Thompson M, Thio M, Cornette L, Ossuetta I, Iglesias I, Theyskens C, de Jong M, Gill B, Ahluwalia JS, de Zegher F, Dunger DB. Prevalence and determinants of hyperglycemia in very low birth weight infants: cohort analyses of the NIRTURE study. J Pediatr. 2010;157:715-719. Epub 2010 Jun 8
2. Ditzenberger GR, Collins SD, Binder N. Continuous insulin intravenous infusion therapy for VLBW infants. J Perinat Neonatal Nurs. 1999;13:70-82.
3. Zamir I, Tornevi A, Abrahamsson T, Ahlsson F, Engström E, Hallberg B, Hansen-Pupp I, Sjöström ES, Domellöf M. Hyperglycemia in Extremely Preterm Infants-Insulin Treatment, Mortality and Nutrient Intakes. J Pediatr. 2018;200:104-110. Epub 2018 May 3
4. Alexandrou G, Skiöld B, Karlén J, Tessma MK, Norman M, Adén U, Vanpée M. Early hyperglycemia is a risk factor for death and white matter reduction in preterm infants. Pediatrics. 2010;125:584-591. Epub 2010 Feb 22
5. Segerer H, Bührer C, Kapellen T, Mattern E, Ramsauer B, Somville T, Trotter A. Betreuung von Neugeborenen diabetischer Mütter. Leitlinie der GNPI, DGPM, DDG, DGHWi, dem DHV, der DGKJ und DGGG. (2k-Level, AWMF-Leitlinien-Register Nr. 024/006, Juli 2017). Z Geburtshilfe Neonatol. 2018;222:107-114. Epub 2018 Jun 19
6. Beardsall K, Thomson L, Elleri D, Dunger DB, Hovorka R. Feasibility of automated insulin delivery guided by continuous glucose monitoring in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2020;105:279-284. Epub 2019 Aug 9
7. Ogilvy-Stuart AL, Beardsall K. Management of hyperglycaemia in the preterm infant. Arch Dis Child Fetal Neonatal Ed. 2010;95:126-131.
8. Beardsall K, Ogilvy-Stuart AL, Ahluwalia J, Thompson M, Dunger DB. The continuous glucose monitoring sensor in neonatal intensive care. Arch Dis Child Fetal Neonatal Ed. 2005;90:307-310.
9. van der Lugt NM, Smits-Wintjens VE, van Zwieten PH, Walther FJ. Short and long term outcome of neonatal hyperglycemia in very preterm infants: a retrospective follow-up study. BMC Pediatr. 2010;10:52.

10. Szymońska I, Jagła M, Starzec K, Hrnciar K, Kwinta P. The Incidence Of Hyperglycaemia In Very Low Birth Weight Preterm Newborns. Results of A Continuous Glucose Monitoring Study--Preliminary Report. Dev Period Med. 2015;19:305-312.
11. Fernández-Martínez MDM, Gómez-Llorente JL, Momblán-Cabo J, Martin-González M, Calvo-Bonachera M, Olvera-Porcel M, Bonillo-Perales A. Monitoring the incidence, duration and distribution of hyperglycaemia in very-low-birth-weight newborns and identifying associated factors. J Perinat Med. 2020;48:631-637.
12. Ng SM, May JE, Emmerson AJ. Continuous insulin infusion in hyperglycaemic extremely-low- birth-weight neonates. Biol Neonate. 2005;87:269-272. Epub 2005 Feb 4
13. Alsweiler JM, Harding JE, Bloomfield FH. Tight glycemic control with insulin in hyperglycemic preterm babies: a randomized controlled trial. Pediatrics. 2012;129:639-647. Epub 2012 Mar 19
14. Hewson M, Nawadra V, Oliver J, Odgers C, Plummer J, Simmer K. Insulin infusions in the neonatal unit: delivery variation due to adsorption. J Paediatr Child Health. 2000;36:216-220.
15. Wilson D, Mc Clure G. Continuous Insulin Infusion is Safe and Efficacious in the Hyperglycemic ELBW Infant: Results of a RCT † 1587. Pediatr Res. 1999;43(Suppl 4):271.
16. Wintergerst KA, Hargadon S, Hsiang HY. Continuous subcutaneous insulin infusion in neonatal diabetes mellitus. Pediatr Diabetes. 2004;5:202-206.
17. Beardsall K, Pesterfield CL, Acerini CL. Neonatal diabetes and insulin pump therapy. Arch Dis Child Fetal Neonatal Ed. 2011;96:223-224. Epub 2010 Nov 29
18. Dorling J, Abbott J, Berrington J, Bosiak B, Bowler U, Boyle E, Embleton N, Hewer O, Johnson S, Juszczak E, Leaf A, Linsell L, McCormick K, McGuire W, Omar O, Partlett C, Patel M, Roberts T, Stenson B, Townend J; SIFT Investigators Group. Controlled Trial of Two Incremental Milk-Feeding Rates in Preterm Infants. N Engl J Med. 2019;381:1434-1443.
19. Paulsen ME, Brown SJ, Satrom KM, Scheurer JM, Ramel SE, Rao RB. Long-Term Outcomes after Early Neonatal Hyperglycemia in VLBW Infants: A Systematic Review. Neonatology. 2021;118:509-521. Epub 2021 Aug 19
20. Caldas JP, Braghini CA, Mazzola TN, Vilela MM, Marba ST. Peri-intraventricular hemorrhage and oxidative and inflammatory stress markers in very-low birth weight newborns. J Pediatr (Rio J). 2015;91:373-379. Epub 2015 Apr 23
21. Ostertag SG, Jovanovic L, Lewis B, Auld PA. Insulin pump therapy in the very low birth weight infant. Pediatrics. 1986;78:625-630.
22. Meetze W, Bowsher R, Compton J, Moorehead H. Hyperglycemia in extremely- low-birth-weight infants. Biol Neonate. 1998;74:214-221.
23. Kao LS, Morris BH, Lally KP, Stewart CD, Huseby V, Kennedy KA. Hyperglycemia and morbidity and mortality in extremely low birth weight infants. J Perinatol. 2006;26:730-736. Epub 2006 Aug 24
24. Zamir I, Stoltz Sjöström E, Ahlsson F, Hansen-Pupp I, Serenius F, Domellöf M. Neonatal hyperglycaemia is associated with worse neurodevelopmental outcomes in extremely preterm infants. Arch Dis Child Fetal Neonatal Ed. 2021;106:460-466. Epub 2021 Apr 16
25. Knopp Nee Dickson JL, Lynn AM, Shaw GM, Chase JG. Safe and effective glycaemic control in premature infants: observational clinical results from the computerised STAR-GRYPHON protocol. Arch Dis Child Fetal Neonatal Ed. 2019;104:205-211. pub 2018 Jun 21
26. Zhou T, Boettger M, Knopp JL, Lange M, Heep A, Chase JG. Model-based subcutaneous insulin for glycemic control of pre-term infants in the neonatal intensive care unit. Comput Biol Med. 2023;160:106808. Epub 2023 Mar 21

ORIGINAL ARTICLE

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Whole Exome Sequencing Revealed Paternal Inheritance of Obesity-related Genetic Variants in a Family with an Exclusively Breastfed Infant

Hazal Banu Olgun Çelebioğlu[1,2], Ayşe Pınar Öztürk[3], Şükran Poyrazoğlu[3], Feyza Nur Tuncer[1]

[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Turkiye
[2]Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Turkiye
[3]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatrics, Pediatric Endocrinology Unit, Istanbul, Turkiye

What is already known on this topic?

Obesity is a complex disorder characterized by excess body fat that manifests under the influence of genetic and environmental factors. Rapid growth during infancy and early childhood has directly been related to the onset of adult obesity. Whole exome sequencing (WES) has been used for identifying novel rare variants in disease pathogenesis. Nevertheless, the mechanism underlying this complex disease is still incompletely understood.

What this study adds?

WES analysis in combination with family segregation was used in predicting the risk for later obesity of an exclusively breastfed infant, and in providing genetic counseling to the family. The paternal inheritance of all potentially deleterious novel obesity-related variants was confirmed in the family.

Abstract

Objective: Obesity is a serious health problem that progressively affects individuals' lives with comorbidities, such as heart disease, stroke, and diabetes mellitus. Since its prevalence has increased, particularly in children less than five years old, its genetic and environmental causes should be determined for prevention and control of the disease. The aim of this study was to detect underlying genetic risk factors in a family with an exclusively breastfed obese infant.

Methods: A three-generation family was recruited to be evaluated for obesity. Detailed examinations along with body mass index (BMI) calculations were performed on available family members. Whole exome sequencing (WES) was performed on a 7-month-old obese infant. Bioinformatic analyses were performed on the Genomize SEQ platform with variant filtering at minor allele frequencies < 1 % for all normal populations. Sanger sequencing was applied in variant confirmation and family segregation.

Results: Neuro-motor developmental features were normal and genetic syndromes were excluded from the index. Early-onset severe obesity (+ 4.25 standard deviation score weight-for-height) was evident in index case; his father and grandmother were also obese (BMIs 38.1 kg/m^2 and 31.3 kg/m^2, respectively). WES analysis revealed deleterious variants in *SH2B1, PDE11A, ADCY3,* and *CAPN10* genes previously associated with obesity. All variants were evaluated as novel candidates for obesity, except *PDE11A*, and family segregation confirmed paternal inheritance.

Conclusion: This study confirmed the paternal inheritance of all potentially deleterious obesity-related variants. The cumulative effect of individual variants might explain the obesity phenotype in this family. The infant is recommended to be followed up periodically due to increased risk for later childhood obesity.

Keywords: Early-onset obesity, whole exome sequencing, paternal inheritance, novel variants, body mass index

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Address for Correspondence: Feyza Nur Tuncer PhD, Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Department of Genetics, Istanbul, Turkiye
E-mail: ftuncer@istanbul.edu.tr **ORCID:** orcid.org/0000-0001-8233-1839

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Introduction

Obesity is a complex disorder characterized by excess body fat that manifests through the influence of both genetic and environmental factors. Various clinical manifestations of obesity originate from diverse phenotypic expression of genetic variants (1). Polygenic/common obesity is the most prevalent type of obesity in society, which occurs because of the effect of many polymorphisms, each contributing small effects (2). There is an imbalance between energy intake and consumption, which increases adipose tissue due to a combination of genetic predisposition and environmental factors (3). However, rare abnormalities linked to a single gene may occur and are termed monogenic or Mendelian obesity. Early-onset severe obesity is the development of obesity in early life, which might occur due to disruption in genes involved in pathways affecting energy balance, appetite, and adipocyte distribution (4). Obesity increasingly affects children under the age of 5 years, highlighting the importance of the early childhood period (5). According to the World Health Organization (WHO), in 2020 nearly 39 million children under the age of 5 years were reported to be overweight or obese (6). Therefore, if protective and preventive measures are not taken, it is predicted that one-fifth of the world's population will be obese by 2025 (7).

Rapid growth during infancy, especially in the first 4 months, and in early childhood (within the first 2 years of life) have been directly linked with the onset of adult obesity (6,8). Physiologic alterations in this critical developmental period contribute to the risk of obesity and comorbidities observed in adulthood (9). Consequently, infants born large for gestational age, along with rapid early growth and physical inactivity have been identified as significant risk factors for obesity (10,11,12). Meta-analysis comprising twin, family, and adoption studies have reported that the heritability of body mass index (BMI) ranges from 40 % to 70 % , implicating the role of genetics (13,14,15). Moreover, the genetic basis has been reported to influence hyperphagia, the disruption of energy balance, and body weight regulation, which all together may lead to severe pediatric obesity (3). Hence, genetic studies involving children are important to determine the type of obesity, its diagnosis, and the risk of recurrence, as well as in providing genetic counseling (16). In this respect, genome-wide association studies (GWAS) have been used extensively to identify common, risk-conferring alleles, while whole exome sequencing (WES) was allowed the identification of novel rare variants in disease pathogenesis (17,18). GWAS exploits mostly non-coding single nucleotide polymorphisms (SNPs) to associate their impacts on the transcriptional regulation of nearby genes through comparison of cases and controls. Since 85 % of mutations are located in protein-coding regions of the genome, WES has been used for detecting deleterious rare variants in disease pathogenesis (19). Therefore, the GWAS and WES approaches complement each other in deciphering the missing heritability in complex disorders. However, in the case of obesity research, the collective efforts of these approaches have managed to explain only 2 % to 6 % of the genetic component of obesity in association with BMI variation (4,20), highlighting the need for further research in varied populations.

The aim of this study was to identify novel rare variants associated with early childhood obesity in a three-generation family having an exclusively breastfed obese infant. The inheritance of obesity appeared to be paternal. Therefore, WES analysis in combination with family segregation was used in predicting the risk for later obesity of this infant, and in providing genetic counseling to this family.

Methods

Patients and Clinical Assessments

A family from Turkiye, involving an exclusively breastfed, 7-month-old, obese male infant along with his parents and grandmother, was recruited for this study. The index (HP028) was born as the first child of non-consanguineous parents and had abnormal weight gain in the early months of life. The grandmother reported a similar growth pattern for her son, with obesity starting in his early childhood and persisting throughout his life without any comorbidity. She and her deceased spouse were reported to be obese and overweight, respectively, from early childhood. The index was subjected to a detailed clinical examination comprising physical and serological evaluations. Detailed clinical data on family history was collected from the adult recruits. In children younger than 2 years of age, obesity is diagnosed if the sex specific weight for recumbent height is more than 97.7 percentile or 2 standard deviation scores (SDS) according to WHO growth standards (21).

Weight, height, and BMI SDSs of the index case were calculated according to the growth chart prepared with national standards and weight for height SDS was based on WHO growth standards (22,23). BMI values of adult individuals were calculated by height, and weight respectively. Following WHO standards, BMI values greater than 30 kg/m^2 were defined as obese and marked with black color on the pedigree (Figure 1). Written informed consents were obtained from all individuals or legal representatives in accordance with Istanbul University, Istanbul Medical Faculty Clinical Research Ethics Committee (protocol no: 2020/1054, date: 01.09.2020). According to the manufacturer's instructions, DNA was extracted using Purelink Genomic DNA Mini Kit

from peripheral blood (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The quantity and purity of DNA samples were measured with NanoDrop ND2000 (Thermo Fisher Scientific Inc.) spectrophotometry and samples were run on agarose gel as a final quality control.

Whole Exome Sequencing and Familial Segregation

WES was performed in the index case under the service of Izmir Tinaztepe University, Faculty of Medicine, Medical Genetic Diagnostic Center (Izmir, Turkiye). Exonic DNA was captured with the Twist Comprehensive Exome kit (Twist Bioscience, South San Francisco, CA, USA), which was used in library preparation. 36.8 Mb of protein-coding regions covering >99% of RefSeq, CCDS, and GENCODE databases were targeted in this manner. Thereafter, sequencing was performed on the Illumina NextSeq 550 platform to achieve a minimum of 20X reading depth for the targeted bases.

Sequence annotations and variant filtering were conducted on the SEQ platform version 16.7 (https://seq.genomize.com; Genomize Inc., Istanbul, Turkiye), which processes FASTQ files by aligning to the GRCh37/hg19 reference genome with a Burrows-Wheeler Alignment (BWA) tool (24). Variants were selected with Freebayes, after duplicate products and realignments of indels were removed by Genomize's proprietary algorithms (25). Variants were annotated using VEP v102 (26). All variant prioritizations were performed for minor allele frequencies (MAF) <1% in all normal populations to detect rare variants. Initially, a whole variant list was filtered to select obesity-related genes obtained from the literature (Supplementary Table 1). Secondly, intronic and synonymous variants were filtered out. The IGV_2.9.4 program was used to visualize sequence reads. MAFs were obtained from GnomAD, 1000 Genomes Project, Exome Sequencing Project, TopMED, and SEQ-specific cohorts comprising approximately 15,000 exome sequences of individuals from Turkiye with varying disorders. A set of *in silico* prediction tools, including FATHMM, M-CAP, CADD, SIFT4G, DANN, Polyphen-2, and Mutation Taster were used to examine the possible impact of selected variants on protein function. Human Splicing Finder (HSF Pro v3.1, Genomnis SAS Company) was used to evaluate the impact of splice region variants. The Genomic Evolutionary Rate Profiling (GERP) score was employed to estimate the evolutionary constraint in a particular position. Sanger sequencing was used to validate the variations obtained from the WES data and to perform segregation analysis. CLC Main Workbench 8.5 was used in Sanger sequence analysis against the reference sequence of the Ensembl GRCh37.p13 version. The list of primers used in Sanger sequencing are listed in Supplementary Table 2.

Results

Clinical Evaluations

Herein, we describe a family comprising a child with excess weight gain, his parents, and his grandmother (Figure 1). Currently, the BMIs of HP030 and HP031 were calculated

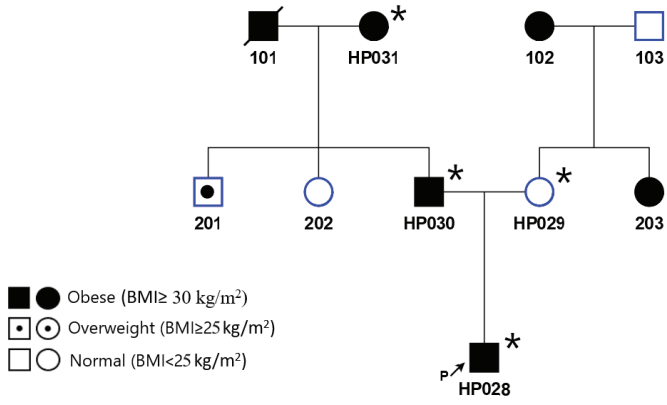

Figure 1. Detailed pedigree of the family with an obese infant. A three-generation family having an exclusively breastfed obese infant was analyzed in terms of obesity. The obese index case (7-month-old, male) was shown by an arrow. Asterisk indicates members with available genetic material. Body weight status was determined by WHO standards

WHO: World Health Organization

Table 1. Details of data quality and filtering results revealed by WES analysis

Quality metrics of whole exome sequencing data	
Total number of reads aligned	69.6M
(M = million)	
Average depth (%)	150.22
% Targets with 50X coverage	99.96
Total number of annotations	235.8K
(K = thousand)	
Total number of variants	35,554
Variants in candidate obesity genes	1,536
(Supplementary Table 1)	
Number of pathogenic variants*	10
Number of likely pathogenic variants*	6
Number of variants of uncertain significance (VUS)*	3,454
Homozygous variants	1,253
Heterozygous variants	23,024
Variant filtering for MAF ≤0.01	2,626

*Pathogenicity determined in accordance with ACMG guidelines.
WES was performed for the index case and exonic DNA was captured with the Twist Comprehensive Exome Kit (Twist Bioscience, South SF, CA, USA). Sequencing was performed on the Illumina Nextseq 550 platform with at least 20X reading depth. Numbers of obtained variants after filtering were shown in the table in different classifications.
WES: whole exome sequencing, VUS: variant of uncertain significance, ACMG: The American College of Medical Genetics and Genomics

Table 2. Details of detected variants in the index case by WES analysis

Gene	Variation	Amino acid change	dbSNP ID	Impact	MAF	Pathogenicity ACMG/SEQ	ClinVar	Mutation taster	SIFT	PolyPhen2	CADD score*	GERP score**
SH2B1	NM_001308293.1:c.28G>A	p.(Gly10Arg)	rs775528324	Missense	0.0000	VUS/VUS+	Not reported	Disease causing	Deleterious	Possibly damaging	23.3	4.46
PDE11A	NM_016953.4:c.919C>T	p.(Arg307Ter)	rs76308115	Stop gained	0.004	VUS/LP	Reported	Disease causing	NA	NA	36	5.47
CAPN10	NM_023083.4:c.84C>A	p.(Cys28Ter)	-	Stop gained	0.0000	LP/LP	Not reported	Disease causing	NA	NA	34	3.44
ADCY3	NM_001320613.2:c.1532C>T	p.(Ser511Leu)	rs139407103	Missense/ splice region	0.0005	LP/VUS	Not reported	Polymorphism	Tolerated	Tolerated	19.6	4.77

*Variants with a score CADD >20 are predicted to be among the 1.0% most deleterious possible substitutions in the human genome.
**GERP score is a measure of sequence conservation across multiple species. A score greater than 2 can be considered as evolutionary constrained.
dbSNP (www.ncbi.nlm.nih.gov/snp/), PolyPhen 2 (www.genetics.bwh.harvard.edu/pph2/).
MAF: minor allele frequency, VUS: variant of uncertain significance, LP: likely pathogenic, NA: not applicable, WES: whole exome sequencing, ACMG: The American College of Medical Genetics and Genomics

as 38.1 kg/m^2 (obese class II) and 31.3 kg/m^2 (obese class I), respectively, and HP031 had comorbidities of type 2 diabetes mellitus (T2DM) and hyperlipidemia. Moreover, the grandfather (#101), who died at the age of 61 due to complications caused by T2DM, was reported to be overweight. In contrast, the index's mother (HP029) was lean, whereas her sibling (#203) and her mother (#102) were both reported to be obese, and #102 was previously diagnosed with hypertension and T2DM.

The index case, HP028, was delivered at the 42nd gestational week by C-section with no other complications. His weight was 4,060 g (1.35 SDS), and his height was 52 cm (0.7 SDS) at delivery. He gained almost 1.5 kg per month as scaled on periodic examinations. He was admitted to our clinic when he was 7 months old, when his height and weight were measured as 74.2 cm (1.64 SDS), and 13.6 kg (4.07 SDS), respectively, and that weight-for-height was 4.25 SDS. His neuro-motor development was normal, and he showed no distinct facial or body features, making the possibility of a genetic syndrome less likely. Biochemical assessments showed no abnormalities in metabolic, thyroid, adrenal, or pituitary hormones. Hepatic ultrasound reported 2 centimeters of growth of the liver at the age of four months, which was within normal limits. During the first year of life, his weight reached 17 kg (4.22 SDS). He is still under yearly follow-up.

Whole Exome Sequencing Results

Quality metrics and filtering results achieved by WES are displayed in Table 1. WES analysis revealed four heterozygous variants in genes previously associated with obesity, which are detailed in Table 2. All variants were evaluated as novel candidates for obesity, except for the one found in *PDE11A*, which was previously associated with obesity cases having high blood pressure (27). These candidate variants confirmed paternal inheritance in family segregation as shown in Figure 2.

Discussion

Obesity is a multifactorial disorder that is influenced by factors such as irregular energy balance, genetic predisposition, a sedentary lifestyle, and socioeconomic status (28). Obesity has become an epidemic and is increasingly observed, especially among pediatric cases, where genetic predisposition and environmental factors lead to obesity persisting into and through adulthood (6,29). Therefore, clinical follow-up to detect postnatal accelerated growth in the first two years of life, which is recognized as a critical period for the development of childhood obesity, may be important in the prevention of obesity and its disparities in adulthood (3,13,30).

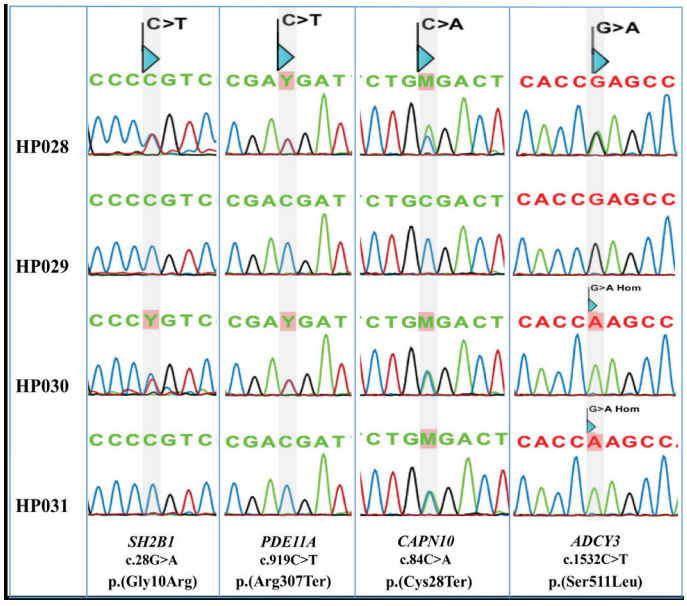

Figure 2. Confirmation of candidate variants by Sanger sequencing and family segregation analysis. Chromatograms of candidate genetic variants are shown in all available family members. Genetic changes are marked with triangles

In this study, we focused on the possible genetic cause of excess weight gain in an obese infant, who was exclusively breastfed. Paternal inheritance was suspected according to the family history, as his father and paternal grandparents were obese from childhood. Results obtained from WES analysis of the index identified four candidate genes that might be responsible for obesity in the members of this family. Among these candidates, two of them are stop-gained, while others are missense variations, as shown in Table 2. Paternal inheritance was confirmed for all these variants through family segregation.

The Src homology 2B adaptor protein 1 *(SH2B1)* p.(Gly10Arg) (rs775528324) novel missense variant was found in the heterozygous state in HP028 and HP030. This gene is involved in body weight regulation as a signaling molecule downstream of the leptin receptor. Disruptions in *SH2B1* were reported to cause anomalies in the digestive system and growth abnormalities, in addition to severe early-onset obesity-insulin resistance syndrome (2). Studies uncovered the causative effects of structural variants or SNPs in this gene as directly associated with increased BMI and severe early-onset obesity (17). According to *in vitro* functional experiments, *SH2B1* has multiple isoforms expressed in a variety of cells in the body. The *SH2B1β* isoform is mainly expressed in the hypothalamus, where body weight is regulated, and it also interacts with the leptin signaling pathway (31). *SH2B1* enhances insulin- and leptin-induced insulin receptor substrate 2 phosphorylation and growth hormone-induced cell motility (32). Despite the many causative variants in *SH2B1* linked to obesity to date, the Gly10Arg variant is described for the first time herein. We suggest that this variant is one of the candidates that might be involved in the obesity phenotype in this family, with high scores gathered from *in silico* predictions for evolutionary conservation by GERP (4.46), and for deleteriousness by CADD (23.30) combined.

In HP028 and HP030, the p.(Arg307Ter) rs76308115 stop-gained variant was detected in the phosphodiesterase 11A *(PDE11A)* gene, a member of the phosphodiesterase (PDE) family of genes. It is a null variant previously associated with obesity that causes loss of function (LOF) and the variant's pathogenicity is predicted to be very strong by *in silico* tools. Along with the Arg307Ter variant (MAF < 0.004), eight more pathogenic null variants in this gene were reported in ClinVar. A GERP score of 5.47 indicates a high conservation pattern for the Arg307 position. PDEs mediate cyclic-AMP (cAMP) degradation to AMP in the cAMP-dependent protein kinase (PKA) signaling pathway, which is involved in the regulation of energy balance through adipogenesis and lipogenesis (33,34). Thus, dysregulation of this pathway has been linked to obesity. Ohlsson et al. (27) previously described the role of *PDE11A* Arg307Ter variant in elevated blood pressure, high BMI, abdominal obesity, and the risk of ischemic stroke, in the Swedish population. Moreover, the Arg307Ter variant has been found in patients diagnosed with pigmented nodular adrenocortical disease and Cushing syndrome, in which obesity can be observed as a component (35,36). Nevertheless, functional validation of this variant is necessary to delineate its role in obesity pathogenicity.

The Adenylate cyclase 3 (*ADCY3)* p.(Ser511Leu) (rs139407103) variant is a novel missense splice-site variant. The impact of this change was reported by Human Splicing Finder (HSF Pro v3.1, Genomnis SAS Company), a splice site predictor tool as a significant alteration of exonic splicing enhancer/exonic splicing silencer motifs ratio. The variant was found to be heterozygous in HP028, while being homozygous in HP030 and HP031. This gene is known to be associated with obesity and BMI Quantitative Trait Locus 19 (BMIQ19), which consists of hyperlipidemia, hyperglyceridemia, and insulin resistance. Adenylate cyclase has a crucial role in the cAMP-dependent PKA signaling pathway by facilitating the production of cAMP from ATP (37). SNPs in the *ADCY3* gene are strongly associated with obesity (38) and it was shown that selective removal of *Adcy3* from the hypothalamus of a mouse led to evident body fat mass gain (39). Saeed et al. (40) suggested that recessive deleterious mutations in *ADCY3* caused monogenic severe obesity, using their data obtained from genetic and functional studies. It

was determined that deep RNA sequencing among homozygous and heterozygous carriers of an *ADCY3* splice-site variation caused severe and intermediate decrement in RNA expression levels, respectively (41). Variations causing splice site disruptions may initiate exon skipping or intron retention, which in turn might impair *ADCY3* function through generating different isoforms. Therefore, the impact of the novel Ser511Leu splice-site variant on both RNA expression and novel isoform generation merits further evaluation.

The Calpain-10 (*CAPN10)* p.(Cys28Ter) stop-gained novel variant was confirmed in HP028, HP030 and HP031. It is a null variant with LOF effects with extremely low frequency in the gnomAD population databases (MAF = 0.0000), which has not previously been reported in ClinVar. Diabetes mellitus, insulin-stimulated glucose uptake, dyslipidemia, adipose tissue disorders, and excess weight gain are known to be associated with *CAPN10*. Functional studies suggest that Calpain-10 is involved in the regulation of glucose homeostasis by participating in the remodeling of the cytoskeleton and catalyzing the translocation of GLUT4 (42). Polymorphisms in this gene were found to play roles in the thermogenesis and beta (3)-adrenoceptor function of obese individuals by reducing lipolytic sensitivity (43). The previously identified C-allele of SNP-44 in *CAPN10* was associated with elevated BMI and obesity, especially in the Chinese population and Turkish T2DM patients (44,45). While particular SNPs were found to regulate lipid metabolism, and lipogenesis in adipocytes, hence contributing to obesity (46), other SNPs were associated with lower BMI rates, particularly in Japanese populations (47). Prior knowledge of this gene concerning obesity along with predictions of LOF by *in silico* tools strongly suggests a role of this variant in the onset of obesity in this family. The results have confirmed the paternal inheritance of all potentially deleterious obesity-related variants. As the functional significance of most of these variants is not fully elucidated, it is presumed that the cumulative effect of these individual SNPs might explain the obesity phenotype observed in this family. Therefore, bearing in mind the father's (HP030) excess weight gain starting in childhood and the paternal inheritance of obesity-related genetic variants detected in this family, genetic counseling was provided to the index (HP028). In this respect, HP028 was predicted to be at increased risk for later obesity, so he should be under regular follow-up accordingly.

Prenatal and perinatal (fetal and early postnatal) influences, such as maternal age and eating habits, the existence of maternal metabolic disorders, intrauterine malnourishment, and even maternal smoking addictions were reported to interact markedly with infant adiposity. Thus, in the intrauterine period, the presence of gestational diabetes mellitus, and insufficient intrauterine nourishment were reported to be causative factors in obesity development (5,48,49). However, interviews with the mother (HP029) informed us of the absence of all these pre- and perinatal risk factors. Her BMI was in normal range (BMI: 22.7 kg/m^2) during and after pregnancy, and her weight gain during pregnancy was within acceptable limits. She did not manifest any metabolic disorders that appeared before, during, or after her labor, nor did she smoke. Hence, we believe that intrauterine risk factors can be excluded for this infant. In terms of postnatal influences, the infant was exclusively breastfed, the fact behind the rationale for this study focusing on delineating genetic components of obesity. However, interviews with the parents revealed that they followed a sedentary life, in which their high BMI and resistance to losing weight could probably have resulted from a combined effect of both lifestyle and the genetic variants they were found to carry.

Study Limitations

Our study is limited since it lacks functional validations, but nevertheless provides some novel, potentially obesity-related genetic variants. So, despite the limitation of small sample size due to inclusion of a single family, the power of detecting rare pathogenic variants, as a result of decreased gene pool, is important in identifying the genetic aspects of complex disorders. Therefore, by investigating a family at high-risk for obesity, we have been able to identify novel genetic variants that have the potential to be the causative variants in this family. However, these suggestions need to be validated, both functionally and by independent cohort studies, in order to assign definite roles to these variants in the pathogenesis of obesity.

Conclusion

Obesity is a complex disorder that involves both genetic and environmental risk factors. Herein, we described three generations of a family having an obese infant, who was exclusively breastfed, suggesting a genetic background for his phenotype and hence the rationale for the study. The clinical and genetic analyses revealed a paternal inheritance of obesity-related variants that may have influenced the condition in this baby. However, the family was advised about the need to exercise regularly in addition to adapting a healthy and balanced diet, due to the complex nature of obesity. Moreover, with the identified genetic risks factors, the infant will undergo regular follow-up assessments to prevent potential health issues. This proactive approach

aims to enable early interventions and personalized care strategies, thereby promoting the child's long-term well-being and development.

Ethics

Ethics Committee Approval: This study was approved by Istanbul University, Istanbul Medical Faculty Clinical Research Ethics Committee (protocol no: 2020/1054, date: 01.09.2020).

Informed Consent: Written and oral informed consents were taken from the family members or legal representatives.

Presented in: The findings of this study were presented as a poster at the European Society of Human Genetics Conference 2023.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayşe Pınar Öztürk, Şükran Poyrazoğlu, Concept: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer, Design: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer, Data Collection or Processing: Hazal Banu Olgun Çelebioğlu, Ayşe Pınar Öztürk, Feyza Nur Tuncer, Analysis or Interpretation: Hazal Banu Olgun Çelebioğlu, Feyza Nur Tuncer, Literature Search: Hazal Banu Olgun Çelebioğlu, Writing: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer.

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References

1. Huvenne H, Dubern B, Clément K, Poitou C. Rare Genetic Forms of Obesity: Clinical Approach and Current Treatments in 2016. Obes Facts. 2016;9:158-173. Epub 2016 Jun 1
2. Loos RJF, Yeo GSH. The genetics of obesity: from discovery to biology. Nat Rev Genet. 2022;23:120-133. Epub 2021 Sep 23
3. Littleton SH, Berkowitz RI, Grant SFA. Genetic Determinants of Childhood Obesity. Mol Diagn Ther. 2020;24:653-663. Epub 2020 Oct 1
4. Saeed S, Arslan M, Froguel P. Genetics of Obesity in Consanguineous Populations: Toward Precision Medicine and the Discovery of Novel Obesity Genes. Obesity (Silver Spring). 2018;26:474-484.
5. Brisbois TD, Farmer AP, McCargar LJ. Early markers of adult obesity: a review. Obes Rev. 2012;13:347-367. Epub 2011 Dec 16
6. Organization. WHO. Obesity and overweight. Last Accessed Date: 10.09.2020. Available from: https://www.who.int/ news-room/fact-sheets/detail/obesity-and-overweight
7. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet. 2016;387:1377-1396.
8. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320:967-971.
9. Dietz WH. "Adiposity rebound": reality or epiphenomenon? Lancet. 2000;356:2027-2028.
10. Woo Baidal JA, Locks LM, Cheng ER, Blake-Lamb TL, Perkins ME, Taveras EM. Risk Factors for Childhood Obesity in the First 1,000 Days: A Systematic Review. Am J Prev Med. 2016;50:761-779. Epub 2016 Feb 22
11. Moschonis G, Grammatikaki E, Manios Y. Perinatal predictors of overweight at infancy and preschool childhood: the GENESIS study. Int J Obes (Lond). 2008;32:39-47. Epub 2007 Dec 4
12. Lanigan J. Prevention of overweight and obesity in early life. Proc Nutr Soc. 2018;77:247-256. Epub 2018 May 29
13. Stagi S, Bianconi M, Sammarco MA, Artuso R, Giglio S, de Martino M. New Thoughts on Pediatric Genetic Obesity: Pathogenesis, Clinical Characteristics and Treatment Approach. Adiposity Omics and Molecular Understanding. InTech; 2017.
14. Elks CE, den Hoed M, Zhao JH, Sharp SJ, Wareham NJ, Loos RJ, Ong KK. Variability in the heritability of body mass index: a systematic review and meta-regression. Front Endocrinol (Lausanne). 2012;3:29.
15. Bouchard C. Genetics of Obesity: What We Have Learned Over Decades of Research. Obesity (Silver Spring). 2021;29:802-820.
16. Paolacci S, Pompucci G, Paolini B, Del Ciondolo I, Miggiano GAD, Aquilanti B, Scaramuzza A, Velluti V, Matera G, Gagliardi L, Bertelli M. Mendelian non-syndromic obesity. Acta Biomed. 2019;90:87-89.
17. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, Frayling TM, Hirschhorn J, Yang J, Visscher PM; GIANT Consortium. Meta-analysis of genome-wide association studies for height and body mass index in ~700000 individuals of European ancestry. Hum Mol Genet. 2018;27:3641-3649.
18. Akbari P, Gilani A, Sosina O, Kosmicki JA, Khrimian L, Fang YY, Persaud T, Garcia V, Sun D, Li A, Mbatchou J, Locke AE, Benner C, Verweij N, Lin N, Hossain S, Agostinucci K, Pascale JV, Dirice E, Dunn M; Regeneron Genetics Center; DiscovEHR Collaboration; Kraus WE, Shah SH, Chen YI, Rotter JI, Rader DJ, Melander O, Still CD, Mirshahi T, Carey DJ, Berumen-Campos J, Kuri-Morales P, Alegre-Díaz J, Torres JM, Emberson JR, Collins R, Balasubramanian S, Hawes A, Jones M, Zambrowicz B, Murphy AJ, Paulding C, Coppola G, Overton JD, Reid JG, Shuldiner AR, Cantor M, Kang HM, Abecasis GR, Karalis K, Economides AN, Marchini J, Yancopoulos GD, Sleeman MW, Altarejos J, Della Gatta G, Tapia-Conyer R, Schwartzman ML, Baras A, Ferreira MAR, Lotta LA. Sequencing of 640,000 exomes identifies GPR75 variants associated with protection from obesity. Science. 2021;373:eabf8683.
19. Kina BG, Topbas Selcuki NF, Bahat PY, Usta T, Aydin S, Rahmioglu N, Tuncer FN, Oral E. Whole exome sequencing reveals novel candidate variants for endometriosis utilizing multiple affected members in a single family. Mol Genet Genomic Med. 2024;12:e2312. Epub 2023 Nov 27
20. Vourdoumpa A, Paltoglou G, Charmandari E. The Genetic Basis of Childhood Obesity: A Systematic Review. Nutrients. 2023;15:1416.
21. Grummer-Strawn LM, Reinold C, Krebs NF; Centers for Disease Control and Prevention (CDC). Use of World Health Organization and CDC growth charts for children aged 0-59 months in the United States. MMWR Recomm Rep. 2010;59:1-15. Erratum in: MMWR Recomm Rep. 2010;59:1184.

22. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.

23. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu MM, Akın MA, Çoban D, Gökoğlu S, Baştuğ O. Body weight, length and head circumference at birth in a cohort of Turkish newborns. J Clin Res Pediatr Endocrinol. 2012;4:132-139. Epub 2012 May 4

24. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754-1760. Epub 2009 May 18

25. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. arXiv. 2016;1207.

26. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. The Ensembl Variant Effect Predictor. Genome Biol. 2016;17:122.

27. Ohlsson T, Lindgren A, Engström G, Jern C, Melander O. A stop-codon of the phosphodiesterase 11A gene is associated with elevated blood pressure and measures of obesity. J Hypertens. 2016;34:445-451.

28. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne). 2021;12:706978.

29. Kelly AS. Current and future pharmacotherapies for obesity in children and adolescents. Nat Rev Endocrinol. 2023;19:534-541. Epub 2023 Jun 19

30. Díaz-Rodríguez M, Pérez-Muñoz C, Lendínez-de la Cruz JM, Fernández-Gutiérrez M, Bas-Sarmiento P, Ferriz-Mas BC. Effectiveness of a Multifactorial Intervention in the First 1000 Days of Life to Prevent Obesity and Overweight in Childhood: Study Protocol. Int J Environ Res Public Health. 2020;17:2239.

31. Ren D, Zhou Y, Morris D, Li M, Li Z, Rui L. Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. J Clin Invest. 2007;117:397-406. Epub 2007 Jan 18

32. Giuranna J, Volckmar AL, Heinen A, Peters T, Schmidt B, Spieker A, Straub H, Grallert H, Müller TD, Antel J, Haußmann U, Klafki H, Rui L, Hebebrand J, Hinney A. The Effect of SH2B1 Variants on Expression of Leptin- and Insulin-Induced Pathways in Murine Hypothalamus. Obes Facts. 2018;11:93-108. Epub 2018 Apr 10 Erratum in: Obes Facts. 2018;11:176-180.

33. London E, Bloyd M, Stratakis CA. PKA functions in metabolism and resistance to obesity: lessons from mouse and human studies. J Endocrinol. 2020;246:51-64.

34. Tong T, Shen Y, Lee HW, Yu R, Park T. Adenylyl cyclase 3 haploinsufficiency confers susceptibility to diet-induced obesity and insulin resistance in mice. Sci Rep. 2016;6:34179.

35. Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, Stein E, Levine E, Delimpasi G, Hsiao HP, Keil M, Heyerdahl S, Matyakhina L, Libè R, Fratticci A, Kirschner LS, Cramer K, Gaillard RC, Bertagna X, Carney JA, Bertherat J, Bossis I, Stratakis CA. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. Nat Genet. 2006;38:794-800. Epub 2006 Jun 11

36. Libé R, Horvath A, Vezzosi D, Fratticci A, Coste J, Perlemoine K, Ragazzon B, Guillaud-Bataille M, Groussin L, Clauser E, Raffin-Sanson ML, Siegel J, Moran J, Drori-Herishanu L, Faucz FR, Lodish M, Nesterova M, Bertagna X, Bertherat J, Stratakis CA. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. J Clin Endocrinol Metab. 2011;96:208-214. Epub 2010 Nov 3

37. Wu L, Shen C, Seed Ahmed M, Östenson CG, Gu HF. Adenylate cyclase 3: a new target for anti-obesity drug development. Obes Rev. 2016;17:907-914. Epub 2016 Jun 3

38. Tian Y, Peng B, Fu X. New ADCY3 Variants Dance in Obesity Etiology. Trends Endocrinol Metab. 2018;29:361-363. Epub 2018 Feb 14

39. Cao H, Chen X, Yang Y, Storm DR. Disruption of type 3 adenylyl cyclase expression in the hypothalamus leads to obesity. Integr Obes Diabetes. 2016;2:225-228. Epub 2016 May 13

40. Saeed S, Bonnefond A, Tamanini F, Mirza MU, Manzoor J, Janjua QM, Din SM, Gaitan J, Milochau A, Durand E, Vaillant E, Haseeb A, De Graeve F, Rabearivelo I, Sand O, Queniat G, Boutry R, Schott DA, Ayesha H, Ali M, Khan WI, Butt TA, Rinne T, Stumpel C, Abderrahmani A, Lang J, Arslan M, Froguel P. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. Nat Genet. 2018;50:175-179. Epub 2018 Jan 8

41. Grarup N, Moltke I, Andersen MK, Dalby M, Vitting-Seerup K, Kern T, Mahendran Y, Jørsboe E, Larsen CVL, Dahl-Petersen IK, Gilly A, Suveges D, Dedoussis G, Zeggini E, Pedersen O, Andersson R, Bjerregaard P, Jørgensen ME, Albrechtsen A, Hansen T. Loss-of-function variants in ADCY3 increase risk of obesity and type 2 diabetes. Nat Genet. 2018;50:172-174. Epub 2018 Jan 8

42. Turner MD, Cassell PG, Hitman GA. Calpain-10: from genome search to function. Diabetes Metab Res Rev. 2005;21:505-514.

43. Hoffstedt J, Näslund E, Arner P. Calpain-10 gene polymorphism is associated with reduced beta(3)-adrenoceptor function in human fat cells. J Clin Endocrinol Metab. 2002;87:3362-3367.

44. Chen SF, Lu XF, Yan WL, Huang JF, Gu DF. Variations in the calpain-10 gene are associated with the risk of type 2 diabetes and hypertension in northern Han Chinese population. Chin Med J (Engl). 2007;120:2218-2223.

45. Demirci H, Yurtcu E, Ergun MA, Yazici AC, Karasu C, Yetkin I. Calpain 10 SNP-44 gene polymorphism affects susceptibility to type 2 diabetes mellitus and diabetic-related conditions. Genet Test. 2008;12:305-309.

46. Pánico P, Salazar AM, Burns AL, Ostrosky-Wegman P. Role of calpain-10 in the development of diabetes mellitus and its complications. Arch Med Res. 2014;45:103-115. Epub 2014 Feb 4

47. Shima Y, Nakanishi K, Odawara M, Kobayashi T, Ohta H. Association of the SNP-19 genotype 22 in the calpain-10 gene with elevated body mass index and hemoglobin A1c levels in Japanese. Clin Chim Acta. 2003;336:89-96.

48. Larqué E, Labayen I, Flodmark CE, Lissau I, Czernin S, Moreno LA, Pietrobelli A, Widhalm K. From conception to infancy - early risk factors for childhood obesity. Nat Rev Endocrinol. 2019;15:456-478. Epub 2019 Jul 3

49. Oken E, Gillman MW. Fetal origins of obesity. Obes Res. 2003;11:496-506.

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ORIGINAL ARTICLE

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Frequency of Delayed Puberty in Boys with Contemporary Management of Duchenne Muscular Dystrophy

Sarah McCarrison[1], Melissa Denker[1], Jennifer Dunne[2], Iain Horrocks[2], Jane McNeilly[3], Shuko Joseph[2], Sze Choong Wong[1,4]

[1]Royal Hospital for Children, Clinic of Paediatric Endocrinology, Glasgow, United Kingdom
[2]Royal Hospital for Children, Clinic of Paediatric Neurology, Glasgow, United Kingdom
[3]Queen Elizabeth University Hospital, Department of Biochemistry, Glasgow, United Kingdom
[4]University of Glasgow, Department of Human Nutrition, Glasgow, United Kingdom

What is already known on this topic?

Delayed puberty represents a documented clinical outcome associated with the prolonged administration of glucocorticoids in adolescents with Duchenne muscular dystrophy (DMD). Nevertheless, there exists a dearth of comprehensive data regarding the prevalence and severity of delayed puberty in individuals affected by DMD.

What this study adds?

Based on clinical assessments conducted by a paediatric endocrinologist, delayed puberty was observed in a striking 82% of boys with DMD who were receiving daily glucocorticoids. Our findings underscore the critical importance of implementing regular puberty monitoring and managing delayed puberty in adherence to international standards of care. Moreover, these results offer a benchmark for evaluating the efficacy of newer treatment strategies designed to mitigate glucocorticoid-related side effects.

Abstract

Objective: Delayed puberty is thought to be common in boys with Duchenne muscular dystrophy (DMD) treated with long term oral glucocorticoid. The aim of this study was to report the frequency of delayed puberty in DMD from examination by a paediatric endocrinologist alongside detailed endocrine investigations.

Methods: All boys with DMD aged at least 14 years in January 2022 known to the paediatric neuromuscular service (2016-2022) were included. Delayed puberty was defined based on testicular volume and genital staging in comparison to a published puberty nomogram.

Results: Twenty-four out of 37 boys (65%) had evidence of delayed puberty and 23/24 (96%) were on glucocorticoid therapy, all of whom were on daily glucocorticoid. However, 7/13 (54%) with normal timing of puberty were on glucocorticoid; 2/7 (29%) were on the intermittent regimen. Of those who were on daily glucocorticoid therapy at the time of assessment of puberty, 23/28 (82%) had evidence of delayed puberty. In boys with delayed puberty, endocrine investigations showed low luteinizing hormone with undetectable testosterone levels, a pre-pubertal response with lutenizing hormone releasing hormone test and sub-optimal testosterone levels with prolonged human chorionic gonadotropin stimulation.

Conclusion: The frequency of delayed puberty in boys with DMD was 65%. Eighty-two percent of adolescent boys with DMD on daily glucocorticoid had evidence of delayed puberty. Biochemical investigations point to functional central hypogonadism in these adolescents. Our data supports the routine monitoring of puberty in boys with DMD.

Keywords: Delayed puberty, hypogonadism, glucocorticoid, deflazacort, prednisolone

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Address for Correspondence: Sze Choong Wong MD, Royal Hospital for Children, Clinic of Paediatric Endocrinology; University of Glasgow, Department of Human Nutrition, Glasgow, United Kingdom
E-mail: jarod.wong@glasgow.ac.uk **ORCID:** orcid.org/0000-0003-2845-6555

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Introduction

Duchenne muscular dystrophy (DMD) is a rare, X-linked muscular dystrophy affecting approximately 1 in 3500 boys due to a mutation in the dystrophin gene. Despite advances in research in DMD, there is currently still no curative therapy for people with DMD (1). Long-term oral glucocorticoid is accepted worldwide as disease modifying therapy and has been a standard of care for over two decades (2). Glucocorticoid is generally initiated between 4-7 years and continued indefinitely into adulthood with proven benefits on skeletal muscle and cardiorespiratory outcomes and may also play a role in reducing early mortality (3). The most common glucocorticoid regimens used worldwide are daily deflazacort, daily prednisolone and intermittent (10-days on/off) prednisolone. A recent international clinical trial confirmed the results of previous studies and smaller clinical trials, demonstrating the superiority of daily glucocorticoid (deflazacort and prednisolone) on skeletal muscle outcomes following three years of therapy compared with 10-days on/off prednisolone (4). However, published studies also demonstrate a higher glucocorticoid side effect burden in those treated with daily glucocorticoid, especially for endocrine consequences, such as growth failure, weight gain and bone fragility (5,6). Further information on the side effects of glucocorticoid in DMD is of great benefit for families to understand the risk versus benefits of long-term treatment.

Another known consequence of long-term glucocorticoid is its impact on pubertal development (7,8). Delayed puberty can contribute to the psychological distress in these adolescents who are already markedly different from their peers due to the short stature, obesity and cushingoid features, in addition to the physical limitations. Fragility fractures are also very common with clinical fractures reported in at least 50% of glucocorticoid treated boys by the age of 11 years (9). With progression of normal puberty in healthy adolescents, bone accrual increases by about 40% (10). Therefore, delayed puberty in DMD is postulated to be an additional insult to the skeleton. However, there are very limited studies on pubertal delay in boys with DMD.

The aim of this study was to report on the frequency of delayed puberty in our whole clinic cohort of boys with DMD as evaluated by clinical examination by a paediatric endocrinologist and biochemical assessments of the hypothalamic-pituitary-gonadal axis.

Methods

Patients

Since January 2016, all boys with DMD who were at least 13 years of age managed in the paediatric neuromuscular clinic in the Royal Hospital for Children, Glasgow were referred to the paediatric endocrine clinic for assessment of puberty, if not already known to the endocrine service. All eligible adolescents were reviewed in the paediatric endocrine clinic. Thirty-eight boys were aged 14 years or older in January 2022 and had examination of puberty at least twice. One boy was excluded due to a concurrent diagnosis of neurofibromatosis type-1 with optic glioma where early puberty can occur. A total of 37 boys are included in this report.

Pubertal Assessment

Assessment of puberty was performed by a single paediatric endocrinologist, which included clinical evaluation of testicular volume and genitalia stage (11). Pubertal assessment was performed when the young person was recumbent, where possible. Based on testicular volume and genitalia stage in comparison with a published puberty nomogram, each boy was classified as having evidence of delayed puberty [<-2.0 standard deviation (SD) below the mean for age] or "normal" timing of puberty (12). We used this method of classification rather than the traditional definition of delayed puberty (testicular volume <4 mL by 14 years) as not all boys were reviewed exactly at 14 years of age. For boys who were treated with testosterone therapy, clinical examination prior to initiation of therapy was used for purposes of classification. For boys who did not receive testosterone therapy, either the latest clinical examination or when testicular volume was first noted to be <4 mL was used for purposes of classification. For boys with bilateral impalpable testes, testicular ultrasound volume in comparison with published paediatric reference data was used for classification, with boys classified as having evidence of delayed puberty if testicular ultrasound volume was <-2.0 SD below the mean for age (13). For boys with single testis or bilateral testes that were impalpable on two occasions despite attempts to milk into the scrotum, testicular ultrasound was performed.

Biochemical Evaluation of Testicular Function

Blood samples were performed which included non-timed luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in accordance with the 2018 international standards of care for DMD (2). Blood samples were mostly conducted in the morning (but not necessarily at 9 am) coinciding with clinical review. Testosterone was measured using liquid chromatography-tandem mass spectrometry via the Xevo TQS tandem mass spectrometer (Waters Corporation, Milford, MA, USA) with an assay sensitivity of 0.5 nmol/L. Plasma samples were extracted using Biotage supported liquid extraction, automated on the CTC PAL

(MicroLiter Analytical Supplies Inc, Suwanne, Georgia, USA), followed by ultra-performance liquid chromatographic separation. For this, assay sensitivity was 0.1 nmol/L and the intra- and inter-assay CV for this assay was <8%. FSH and LH were measured by two-step chemiluminescent microparticle immunoassay on the Abbott Architect (Abbott Laboratories, IL, USA). The functional sensitivities were 0.2 mU/L and the intra – and inter-assay coefficients were <5%.

Luteinizing Hormone Releasing Hormone and Prolonged Human Chorionic Gonadotrophin Stimulation

Between 2016 and 2017, boys with DMD with delayed puberty underwent evaluation of the hypothalamic-pituitary-testicular axis which included LH releasing hormone (LHRH) stimulation test and prolonged human chorionic gonadotrophin (HCG) stimulation test (to evaluate the ability of the Leydig cells of the testes to produce testosterone). These were carried out prior to consideration of testosterone as part of clinical care to guide the duration of testosterone therapy in boys with DMD based on published report suggesting that the combined LHRH and prolonged HCG test may discriminate patients with central hypogonadism and self-limiting delayed puberty (14). In addition, the prolonged HCG test has also been used to attempt to facilitate testicular descent in boys with inguinal testes.

Prior to adminisation of gonadorelin 100 microgam intravenously, blood samples for LH and FSH levels were obtained at 9 am and repeated at 30 and 60 minutes. Prolonged HCG was performed as previously described (15). Intramuscular injection of HCG 1500 IU was administered with blood samples collected at baseline and at day 4. Two further HCG injections were administered each week in the following two weeks with the final blood sample collected on day 22. From 2018, investigations prior to consideration of testosterone therapy were performed in accordance with the 2018 international standards of care for DMD, which did not include LHRH and prolonged HCG stimulation tests (2).

Radiographs: Bone Age and Lateral Thoracolumbar Spine Radiographs for Vertebral Fracture Assessment

Bone age was calculated using the automated BoneExpert 3.0 software (Visiana, Denmark) in accordance with the Tanner-Whitehouse TW2 method (16,17). Annual lateral thoracolumbar spine radiographs have been performed since 2015, and information on vertebral fracture was based on clinical radiological reports by consultant paediatric radiologists. For purposes of this report, lateral spine imaging within six months of evaluation of puberty was used.

Conduct of Study and Consent

This report was conducted as a service evaluation and clinical audit against the 2018 international standards of care for DMD in the area of monitoring and management of puberty (2). All investigations were performed as part of routine clinical care, and anonymised data was collected. Formal ethical approval and written informed consent was not required in line with regulations laid out by the United Kingdom National Health Service Health Research Authority (18). This evaluation of service was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Statistical Analysis

Continuous data is presented as median (minimum and maximum). Discrete variables are reported as frequency in percentages [with 95% confidence intervals (CI)]. Patients were divided into groups based on the presence or absence of delayed puberty for the purpose of comparison based on the puberty nomogram. Delayed puberty was defined as <-2 SD score as previously defined (12). Comparison of continuous variables between the group with delayed puberty and normal timing of puberty was performed using the Mann-Whitney test. Comparison of categorical outcomes between the two groups was performed using the Fisher's exact test. A $p < 0.05$ was accepted as statistically significant. Statistics were performed using IBM Statistical Package for the Social Sciences statistics, version 29 (IBM Inc., Armonk, NY, USA).

Results

Based on testicular volume in comparison to the puberty nomogram on clinical examination or testicular ultrasound and genital staging in comparison to the puberty nomogram, 24/37 (65%; 95% CI: 48% to 80%) had evidence of delayed puberty (12).

Clinical Status

Table 1 shows the clinical characteristics of boys with delayed puberty and those with normal timing of puberty at the time of assessment of puberty. Median age of the 24 boys with delayed puberty was 14.3 years (13.6, 16.7) at assessment of puberty. Median age of the 13 boys with normal timing of puberty was 14.0 years (11.8, 16.8).

At assessment of puberty, 29% with delayed puberty were still ambulant whereas this was only noted in 8% of those with normal timing of puberty ($p = 0.216$). Eight percent in the delayed puberty group required assisted ventilation with nocturnal bilevel positive airway pressure (biPAP) due to an obstructive picture. On the other hand, 15% of those with

Table 1. Clinical characteristics in boys with delayed puberty and those with normal timing of puberty

	Delayed puberty (n = 24)	Normal timing of puberty (n = 13)	p value
Age (years)	14.3 (13.6, 16.7)	14.0 (11.8, 16.8)	0.07
Ambulant	7/24 (29%)	1/13 (8%)	0.216
On oral glucocorticoid	23/24 (96%)	7/13 (54%)	0.004*
Daily glucocorticoid	23/23 (100%)	5/7 (71%)	0.048*
- Daily deflazacort	19/23 (83%)	3/7 (43%)	0.06
- Daily prednisolone	4/23 (17%)	2/7 (29%)	0.60
10 day on/10 days off prednisolone	0/23	2/7 (29%)	0.048*
Duration of glucocorticoid therapy (years)	9.3 (4.0, 11.5)	7.0 (3.7, 7.3)	0.002*
Duration of daily glucocorticoid therapy (years)	5.2 (0.8, 10.0)	4.0 (3.7, 6.0)	0.07
Dose of glucocorticoid in prednisolone equivalent (mg/kg/day)	0.24 (0.13, 0.67)	0.26 (0.08, 0.57)	0.61
Severe scoliosis[a]	1/24 (4%)	5/13 (39%)	0.014*
Vertebral fractures[b]	16/24 (67%)	3/13 (23%)	0.017*
Bone age (years)	10.7 (6.6, 14.9)	13.3 (9.4, 17.1)	0.046*

[a]Severe scoliosis is defined as Cobbs angle > 20 degrees and/or if required surgery; [b]Vertebral fracture is diagnosed by lateral thoracolumbar spine radiographs within 6 months of assessment of puberty.
Data expressed as median (range)

normal timing of puberty were on nocturnal biPAP, all due to due to neuromuscular weakness (p = 0.601). Only one boy in the delayed puberty group had signficiant scoliosis (defined as Cobbs angle > 20 degrees or had required surgery), whereas this was noted in 39% of those with no evidence of delayed puberty (p = 0.014) (19). Sixty-seven percent in the delayed puberty group had evidence of vertebral fracture, whereas this was only noted in 23% of those with normal timing of puberty (p = 0.017). Ninety-six percent (23/24) in the delayed puberty group were still alive in January 2022, whereas this was noted in 77% (10/13) of those with normal timing of puberty (p = 0.115).

Glucocorticoid Therapy

In the group with delayed puberty, 23/24 (96%) boys were on glucocorticoid, all of whom were on daily glucocorticoid (Table 1). nineteen/twenty three (83%) were on daily deflazacort with 4/23 (17%) on daily prednisolone. One boy had discontinued glucocorticoid in the previous four years. In contrast, 7/13 (54%) boys with normal timing of puberty were on glucocorticoid, 5 of whom were on daily glucocorticoid. Of these seven 3 (43%) were on daily deflazacort, 2 (29%) were on daily prednisolone and 2 (29%) were on intermittent (10 days on/10 days off prednisolone). Six of the thirteen boys (46%) with normal timing of puberty were not on glucocorticoid at assessment of puberty, having discontinued glucocorticoid for a median of 2.9 (1.0, 6.8) years. The percentage of boys on glucocorticoid at time of assessment of puberty was significantly higher in the group with delayed puberty compared with the group with normal timing of puberty (96% vs 54%, p = 0.004).

Glucocorticoid Dosing

In the group with delayed puberty and who were on glucocorticoid, median dose of glucocorticoid was 0.24 (0.13, 0.67) mg/kg/day in prednisolone equivalent (Table 1). Of those who were on glucocorticoid in the group with normal timing of puberty, median dose of glucocorticoid was 0.26 (0.08, 0.57) mg/kg/day in prednisolone equivalent. In the group with delayed puberty, median duration of glucocorticoid exposure was 9.3 (4.0, 11.5) years whereas median glucocorticoid exposure was 7.0 (3.7, 7.3) years in the group with normal timing of puberty (p = 0.002). In the group with delayed puberty, 7/23 (30%) were on daily glucocorticoid since initiation whilst the others (16/23 - 70%) were previously on the 10 days on/10 days off regimen. In the group with normal timing of puberty, 2/5 (40%) were on daily glucocorticoid since initiation whilst the others (3/5, 60%) were previously on the 10 days on/10 days off regimen. Median duration of exposure to daily glucocorticoid was 5.2 (0.8, 10.0) years in the boys with delayed puberty whereas median duration of exposure to daily glucocorticoid was 4.0 (3.7, 6.0) years in the boys with normal timing of puberty (p = 0.07).

Delayed Puberty in Boys on Daily Glucocorticoid and 10 Days on/10 Days Off Glucocorticoid

Of the 28 on daily glucocorticoid at the time of assessment of puberty, 23 (82%; 95% CI: 63% to 94%) had evidence of delayed puberty. Neither of the two boys on 10 days on/10 days off glucocorticoid at time of assessment of puberty had evidence of delayed puberty. The percentage of boys on daily glucocorticoid was significantly higher in the group

with delayed puberty compared with the group with normal timing of puberty (100% vs. 71%, p = 0.048).

Delayed Puberty in Boys on Daily Deflazacort and Daily Prednisolone

Of the 21 boys on daily deflazacort, 19 (91%; 95% CI: 70% to 99%) had evidence of delayed puberty (Table 1). Of the 6 boys on daily prednisolone, 4 (67%; 95% CI: 22% to 96%) had evidence of delayed puberty.

Pubertal Assessment in Boys with Delayed Puberty

Twenty-two of the 24 boys (88%) with delayed puberty had testicular volume < 4 mL (combined testicular volume < 8 mL). Sixteen of these 21 (76%) were aged 14 years or older. Two out of the 24 boys (8%) in the delayed puberty group had clinical signs of puberty with genitalia stage 2, pubic hair stage 2, testicular volume 4 mL (combined testicular volume 8 mL) at 15.6 and 16.7 years, respectively, but were classified as having delayed puberty in accordance to the definition adopted for this study based on the puberty nomogram (12). Three boys (13%) with delayed puberty had bilateral testes or single testis in a non-scrotal location: bilateral inguinal testes (n = 2), and left inguinal testis (n = 1). There was no past history of neonatal and childhood undescended testes or family history of undescended testes. Twenty three out of the 24 boys were initiated on testosterone therapy at a median of 14.3 (13.6, 16.7) years in line with the 2018 international standards of care (2). All 23 boys were initiated on an escalating regime of testosterone therapy in accordance with the British Society of Paediatric Endocrinology and Diabetes testosterone replacement therapy guidance (20). Twelve boys were treated with intramuscular testosterone, eight with topical testosterone and three with oral testosterone. A total of six of the 23 boys were initiated on testosterone < 14 years at a median age of 13.7 (13.6, 13.8) years, all of whom were pre-pubertal in line with the current 2018 international standards of care which state that testosterone can be considered from the age of 12 years (2). One boy with delayed puberty was not treated as the decision was made to discontinue glucocorticoid treatment when delayed puberty was identified on clinical examination.

Pubertal Assessment in Boys with Normal Timing of Puberty

In the group with normal timing of puberty (median age 14.0 years), 9/13 boys (69%) were in early to mid-puberty: genitalia stage 2 or 3 and testicular volume of 4-10 mL (combined testicular volume 8-20 mL), whereas four others were in late puberty: genitalia stage 4 or 5 and testicular volume of 12-20 mL (combined testicular volume 24-40 mL). Median testicular volume was 6 (4, 20) mL with median combined testicular volume of 12 mL. All boys in this group had bilateral testes in the scrotum on clinical examination. In ten boys, follow-up clinical examination of puberty was available at a median of 15.8 (14.0, 18.8) years. All ten boys showed further progression of puberty. Seven out of 10 achieved adult external virilisation for genitalia and pubic hair staging (i.e. genitalia stage 5, pubic hair stage 5) at follow-up examination. At follow-up examination, median testicular volume for the ten boys was 15 (8, 20) mL with median combined testicular volume of 30 mL. Of the seven who reached adult external virilization at follow-up clinical examination of puberty, all had testicular volume of at least 15 mL.

Endocrine Investigations

Twenty boys in the delayed puberty group and ten boys in the normal timing of puberty group has LH, FSH and testosterone measured within six months of clinical evaluation of puberty. Nineteen out of 20 (95%) boys with delayed puberty had undetectable LH levels (< 0.2 U/L) with one boy in this group with a measurable LH of 0.4 IU/L. All 10 boys with normal timing of puberty had detectable LH level with a median of 0.8 (0.2, 4.2) IU/L. There was a significant difference between the proportion of boys with undetectable LH in the group with delayed puberty and normal timing of puberty (p < 0.0001). Median FSH in the boys with delayed puberty was 1.1 (0.2, 4.2) IU/L whereas this was 3.0 IU/L in the group with normal timing of puberty (p = 0.03). All 20 boys with delayed puberty had undetectable testosterone levels (< 0.5 nmol/L), whereas all 10 in the group with normal timing of puberty had detectable testosterone levels at a median of 2.4 (0.6, 13.4) nmol/L. There was a significant difference between the proportion of boys with undetectable testosterone in the group with delayed puberty and normal timing of puberty (p < 0.0001).

Six boys with delayed puberty had LHRH stimulation test and prolonged HCG test and results are presented in Figure 1. All boys had an undetectable LH level at baseline which rose to a median peak of 1.4 (0.7, 4.4) IU/L. Peak FSH level was higher than peak LH level in all six boys. All had undetectable testosterone levels at baseline of HCG test which rose to a median of 3.0 (1.5, 5.3) nmol/L on day 4. Three out of the six had testosterone levels > 3.5 nmol/L at day 4. By day 22, 4/6 had undetectable testosterone levels. The fourth boy had testosterone level of 2.3 nmol/L at day 4 and 0.5 nmol/L at day 22. The sixth boy had testosterone level of 3.6 nmol/L at day 4 and 5.6 nmol/L at day 22. In both the boys with bilateral inguinal testes, prolonged HCG stimulation did not lead to testicular descent.

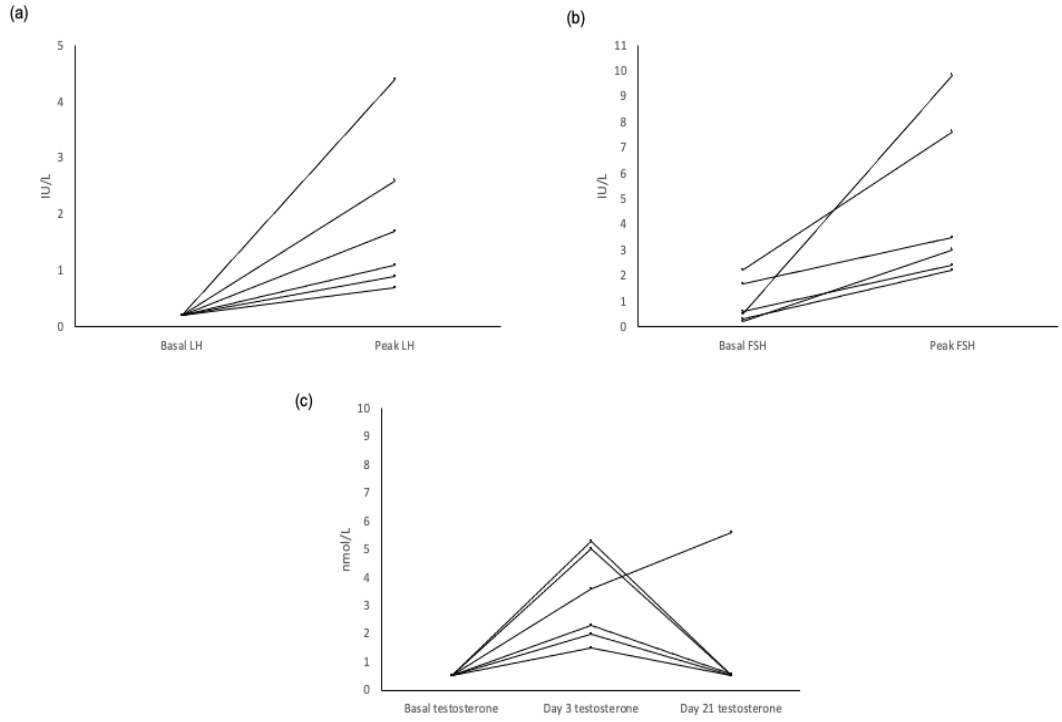

Figure 1. LHRH and prolonged HCG stimulation test in six boys with DMD. a) LH response to LHRH stimulation test. b) FSH response to LHRH stimulation test. c) Testosterone response to prolonged HCG stimulation test

LHRH stimulation test: A peak LH < 5 IU/L to LHRH stimulation and peak FSH > peak LH denotes a pre-pubertal response.

HCG stimulation test: Testosterone levels > 3.5 nmol/L at day 4 and > 9.5 nmol/L at day 22 denotes normal testosterone production.

LHRH: luteinizing hormone-releasing hormone, HCG: human chorionic gonadotrophin, DMD: Duchenne muscular dystrophy, LH: luteinizing hormone, FSH: follicle stimulation hormone

Discussion

This current report identified that delayed puberty was noted in about 65% (upper limit of 95% CI of 80%) of our entire cohort of boys with DMD and in 82% (upper limit of 95% CI of 94%) of those on daily glucocorticoid. Our results provide strong supportive evidence for routine monitoring of puberty, as recommended by the 2018 international standards of care (2). Our results and methods for classification of delayed puberty could be used to study the impact of newer treatment strategies in DMD and pubertal delay, especially those that may be postulated to have less glucocorticoid-associated side effects. Our study also identified abnormal testes location in a small number of boys but only in the group with delayed puberty (13%). The reasons for this are unclear but could point to a state of functional central hypogonadism although further studies are needed.

Current published studies show that 50-97% of adolescents with DMD are pre-pubertal (9,21,22). However, some of these studies included younger boys (5-9 years) where onset of puberty is not expected (21,22). Information on puberty in one study was from self-assessment, which has never been validated in this group of boys (22). Our previous retrospective report focusing on fractures in boys with DMD in all neuromuscular centres in Scotland managed up to December 2015 identified that 79% (11/14) of adolescents aged 14 years or older had evidenced of delayed puberty (9). However, in that previous report, only 48% (14/29) of eligible boys aged 14 years or older had examination of puberty following referral to paediatric endocrinology due to clinical concerns. In our present study, we report puberty in the whole cohort of boys with DMD from the clinic in Glasgow following a clinical pathway of routine assessment of puberty during adolescence since 2016, thereby allowing information on frequency of delayed puberty (65%) in the neuromuscular clinic in Glasgow.

The impact of glucocorticoid regimen on pubertal development in DMD has not been previously evaluated. Published evidence shows that skeletal morbidity, growth

failure and weight gain are more common in boys with DMD treated with daily glucocorticoid in comparison with those on intermittent therapy (5,6). Our study shows the significant side-effect toxicity of daily glucocorticoid on pubertal development as just over 80% of boys with delayed puberty were on daily glucocorticoid therapy, whereas all on 10 days on/10 days off glucocorticoid had no evidence of delayed puberty. Of note, a report in four boys with DMD managed with daily prednisolone for the first two weeks followed by alternate day prednisolone for three years, before changing to alternate day deflazacort reported delayed puberty (23). Thus, larger studies on the impact of intermittent glucocorticoid regimen and puberty are needed. Other factors, such as glucocorticoid type, dose and duration of glucocorticoid treatment may also impact on puberty. Whilst our results show that 91% of boys on daily deflazacort had delayed puberty in comparison to 67% of boys on daily prednisolone, the proportionately larger number of boys on daily deflazacort in our study do not allow us to make firm conclusions, hence this also warrants further study.

The underlying endocrine abnormalitiy of the hypothalamic-pituitary-gonadal axis in adolescents with chronic conditions on long-term glucocorticoid is speculated to be due to a functional state of hypothalamic (central) hypogonadism (8,24). Our endocrine investigations provide supportive evidence of that in DMD. LH levels were suppressed (or low) in those with delayed puberty. LH response to LHRH stimulation indicated no evidence of activation of the hypothalamic-pituitary-gonadal axis and was associated with undetectable or low testosterone levels. In our report, with prolonged HCG stimulation, 3/6 (50%) had testosterone levels of >3.5 nmol/L at day 4 but none had testosterone level >9.5 nmol/L at day 22. Previous publications have defined testosterone level of >3.5 nmol/L at day 4 and >9.5 nmol/L at day 22, following HCG stimulation as an acceptable response (25). It is known that testosterone response to HCG stimulation can be poor in people with central hypogonadism (26). Five out of the six boys (83%) in our report had testosterone levels which were lower at day 22 than day 4 including four boys with undetectable testosterone levels at day 22. Previous reports using the same protocol of HCG stimulation in children with undescended testes showed that 2/16 (13%) and 2/12 (17%) had lower testosterone levels at day 22 than day 4 levels although all these children in the two previous reports still had detectable level of testosterone at day 22 (25,27). We speculate that the pattern of testosterone levels with prolonged HCG could be due to an abnormality of testosterone production due to prolonged gluccorticoid exposure.

Study Limitations

There are limitations to our current study which include the relatively small sample size. Due to the heterogeneity of glucocorticoid treatment, we are unable to conclusively clarify issues like glucocorticoid regimen, dose and type and their impact on delayed puberty. Future studies are needed including a larger group of boys on intermittent glucocorticoid regimen. Future studies should also address pubertal progression in boys with DMD in a standardized research study with regular timing of longitudinal assessments. Nevertheless, our study is the largest report of pubertal delay in adolescents with DMD with complete case ascertainment of all eligible boys in our neuromuscular clinic.

Conclusion

In summary, we report a high frequency of delayed puberty in adolescent boys with DMD, especially in those on daily glucocorticoid. Therefore routine examination of puberty from late childhood in boys with DMD should be mandatory, in line with the 2018 international standards of care (2).

Ethics

Ethics Committee Approval and Informed Consent: Formal ethical approval and written informed consent was not required in line with regulations laid out by the United Kingdom National Health Service Health Research Authority.

Footnotes

Authorship Contributions

Concept: Sarah McCarrison, Melissa Denker, Jennifer Dunne, Iain Horrocks, Jane McNeilly, Shuko Joseph, Sze Choong Wong, Design: Sarah McCarrison, Melissa Denker, Jennifer Dunne, Iain Horrocks, Jane McNeilly, Shuko Joseph, Sze Choong Wong, Data Collection or Processing: Sarah McCarrison, Melissa Denker, Sze Choong Wong, Analysis or Interpretation: Sarah McCarrison, Melissa Denker, Sze Choong Wong, Literature Search: Sarah McCarrison, Sze Choong Wong, Writing: Sarah McCarrison, Sze Choong Wong.

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References

1. Markati T, Oskoui M, Farrar MA, Duong T, Goemans N, Servais L. Emerging therapies for Duchenne muscular dystrophy. Lancet Neurol. 2022;21:814-829. Epub 2022 Jul 15
2. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, Case LE, Clemens PR, Hadjiyannakis S, Pandya S, Street N, Tomezsko

J, Wagner KR, Ward LM, Weber DR; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol. 2018;17:251-267. Epub 2018 Feb 3 Erratum in: Lancet Neurol. 2018;17:495.

3. McDonald CM, Henricson EK, Abresch RT, Duong T, Joyce NC, Hu F, Clemens PR, Hoffman EP, Cnaan A, Gordish-Dressman H; CINRG Investigators. Long-term effects of glucocorticoids on function, quality of life, and survival in patients with Duchenne muscular dystrophy: a prospective cohort study. Lancet. 2018;391:451-461. Epub 2017 Nov 22
4. Guglieri M, Bushby K, McDermott MP, Hart KA, Tawil R, Martens WB, Herr BE, McColl E, Speed C, Wilkinson J, Kirschner J, King WM, Eagle M, Brown MW, Willis T, Griggs RC; FOR-DMD Investigators of the Muscle Study Group; Straub V, van Ruiten H, Childs AM, Ciafaloni E, Shieh PB, Spinty S, Maggi L, Baranello G, Butterfield RJ, Horrocks IA, Roper H, Alhaswani Z, Flanigan KM, Kuntz NL, Manzur A, Darras BT, Kang PB, Morrison L, Krzesniak-Swinarska M, Mah JK, Mongini TE, Ricci F, von der Hagen M, Finkel RS, O'Reardon K, Wicklund M, Kumar A, McDonald CM, Han JJ, Joyce N, Henricson EK, Schara-Schmidt U, Gangfuss A, Wilichowski E, Barohn RJ, Statland JM, Campbell C, Vita G, Vita GL, Howard JF Jr, Hughes I, McMillan HJ, Pegoraro E, Bello L, Burnette WB, Thangarajh M, Chang T. Effect of Different Corticosteroid Dosing Regimens on Clinical Outcomes in Boys With Duchenne Muscular Dystrophy: A Randomized Clinical Trial. JAMA. 2022;327:1456-1468.
5. Joseph S, Wang C, Bushby K, Guglieri M, Horrocks I, Straub V, Ahmed SF, Wong SC; UK NorthStar Clinical Network. Fractures and Linear Growth in a Nationwide Cohort of Boys With Duchenne Muscular Dystrophy With and Without Glucocorticoid Treatment: Results From the UK NorthStar Database. JAMA Neurol. 2019;76:701-709. Erratum in: JAMA Neurol. 2019;76:701-709.
6. Crabtree NJ, Adams JE, Padidela R, Shaw NJ, Högler W, Roper H, Hughes I, Daniel A, Mughal MZ. Growth, bone health & ambulatory status of boys with DMD treated with daily vs. intermittent oral glucocorticoid regimen. Bone. 2018;116:181-186. Epub 2018 Jul 25
7. Wood CL, Straub V, Guglieri M, Bushby K, Cheetham T. Short stature and pubertal delay in Duchenne muscular dystrophy. Arch Dis Child. 2016;101:101-106. Epub 2015 Jul 3
8. Kao KT, Denker M, Zacharin M, Wong SC. Pubertal abnormalities in adolescents with chronic disease. Best Pract Res Clin Endocrinol Metab. 2019;33:101275.
9. Joseph S, Wang C, Di Marco M, Horrocks I, Abu-Arafeh I, Baxter A, Cordeiro N, McLellan L, McWilliam K, Naismith K, Stephen E, Ahmed SF, Wong SC. Fractures and bone health monitoring in boys with Duchenne muscular dystrophy managed within the Scottish Muscle Network. Neuromuscul Disord. 2019;29:59-66. Epub 2018 Sep 25
10. Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. Arch Dis Child. 2005;90:373-378.
11. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45:13-23.
12. Lawaetz JG, Hagen CP, Mieritz MG, Blomberg Jensen M, Petersen JH, Juul A. Evaluation of 451 Danish boys with delayed puberty: diagnostic use of a new puberty nomogram and effects of oral testosterone therapy. J Clin Endocrinol Metab. 2015;100:1376-1385. Epub 2015 Jan 16
13. Joustra SD, van der Plas EM, Goede J, Oostdijk W, Delemarre-van de Waal HA, Hack WW, van Buuren S, Wit JM. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. Acta Paediatr. 2015;104:271-278. Epub 2015 Mar 11
14. Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. J Clin Endocrinol Metab. 2009;94:780-785. Epub 2008 Nov 18
15. Lucas-Herald AK, Kyriakou A, Alimussina M, Guaragna-Filho G, Diver LA, McGowan R, Smith K, McNeilly JD, Ahmed SF. Serum Anti-Müllerian Hormone in the Prediction of Response to hCG Stimulation in Children With DSD. J Clin Endocrinol Metab. 2020;105:1608-1616.
16. Martin DD, Calder AD, Ranke MB, Binder G, Thodberg HH. Accuracy and self-validation of automated bone age determination. Sci Rep. 2022;12:6388.
17. Subramanian S, Viswanathan VK. Bone Age. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2022, StatPearls Publishing LLC.; 2022.
18. Authority HR. HRA Decision Tool: Defining Research. October 2022. Available from: https://www.hra-decisiontools.org.uk/research/docs/DefiningResearchTable_Oct2022.pdf
19. Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, Case LE, Cripe L, Hadjiyannakis S, Olson AK, Sheehan DW, Bolen J, Weber DR, Ward LM; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol. 2018;17:347-361. Epub 2018 Feb 3
20. Chinoy A, Crowne EC, Skae M. BSPED Guideline: Testosterone Therapy in Infancy and Adolescence UK; 2019.
21. Rutter MM, Collins J, Rose SR, Woo JG, Sucharew H, Sawnani H, Hor KN, Cripe LH, Wong BL. Growth hormone treatment in boys with Duchenne muscular dystrophy and glucocorticoid-induced growth failure. Neuromuscul Disord. 2012;22:1046-1056. Epub 2012 Sep 8
22. Annexstad EJ, Bollerslev J, Westvik J, Myhre AG, Godang K, Holm I, Rasmussen M. The role of delayed bone age in the evaluation of stature and bone health in glucocorticoid treated patients with Duchenne muscular dystrophy. Int J Pediatr Endocrinol. 2019;2019:4. Epub 2019 Dec 23
23. Merlini L, Gennari M, Malaspina E, Cecconi I, Armaroli A, Gnudi S, Talim B, Ferlini A, Cicognani A, Franzoni E. Early corticosteroid treatment in 4 Duchenne muscular dystrophy patients: 14-year follow-up. Muscle Nerve. 2012;45:796-802.
24. Mahabadi N, Doucet A, Wong AL, Mahabadi V. Glucocorticoid Induced Hypothalamic-Pituitary Axis Alterations Associated with Hypogonadotropic Hypogonadism Osteology and Rheumatology. 2019.
25. Ahmed SF, Keir L, McNeilly J, Galloway P, O'Toole S, Wallace AM. The concordance between serum anti-Mullerian hormone and testosterone concentrations depends on duration of hCG stimulation in boys undergoing investigation of gonadal function. Clin Endocrinol (Oxf). 2010;72:814-819. Epub 2009 Oct 7
26. Dunkel L, Perheentupa J, Virtanen M, Mäenpää J. Gonadotropin-releasing hormone test and human chorionic gonadotropin test in the diagnosis of gonadotropin deficiency in prepubertal boys. J Pediatr. 1985;107:388-392.
27. Dixon J, Wallace AM, O'Toole S, Ahmed SF. Prolonged human chorionic gonadotrophin stimulation as a tool for investigating and managing undescended testes. Clin Endocrinol (Oxf). 2007;67:816-821. Epub 2007 Jul 20

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Assessment of the Admission and Follow-up Characteristics of Children Diagnosed with Secondary Osteoporosis

Emine Kübra Şen, Merih Berberoğlu, Gizem Şenyazar, Sirmen Kızılcan Çetin, Ayşegül Ceran, Seda Erişen Karaca, Elif Özsu, Zehra Aycan, Zeynep Şıklar

Ankara University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey

What is already known on this topic?

Secondary osteoporosis is a condition when the underlying disease or its treatment causes the bone mass to decrease and the bone structure to deteriorate, increasing the risk of fracture. The importance of diagnosis and treatment during childhood and adolescence is due to the long-term negative effects.

What this study adds?

Secondary osteoporosis is common in children, mostly in chronic inflammatory diseases. Vertebral involvement is common in patients with secondary osteoporosis, even in the absence of a history of significant fracture at the time of diagnosis. The efficacy of bisphosphonate therapy has been demonstrated in patients with secondary osteoporosis with and without a history of steroid drug use.

Abstract

Objective: Secondary osteoporosis is a condition when the underlying disease or its treatment causes the bone mass to decrease and the bone structure to deteriorate, increasing the risk of fracture. The importance of diagnosis and treatment during childhood and adolescence is due to the long-term negative effects. In this study, our objectives were to determine the diagnostic findings, treatment efficacy, and follow-up characteristics of children with secondary osteoporosis.

Methods: Patients diagnosed with secondary osteoporosis between January 2000 and January 2021 were included. The research was a cross-sectional and descriptive study. Study participants had to be under 18 years of age when the primary underlying disease was diagnosed and had received treatment for secondary osteoporosis. Patient data were collected from patient files. Statistical analysis was performed using Statistical Package for the Social Sciences, version 20.0 (IBM Corp, Armonk, NY, USA).

Results: Sixty-one patients (28 female; 45.9%) were evaluated. The most common underlying primary diseases were inflammatory diseases (57.7%), neuromuscular diseases (26.2%), immunodeficiency (13.1%), acute lymphoblastic leukemia (8.2%), metabolic diseases (8.2%), solid organ transplantation (8.2%), bone marrow transplantation (6.6%) and epilepsy (6.6%). The mean ± standard deviation chronological age when secondary osteoporosis was diagnosed was 11.89 ± 4.88 years. Patients were evaluated for osteoporosis at a mean of 6.39 ± 5.13 years after the onset of the underlying primary chronic diseases. Most (78.7%) had a history of one or more chronic drug use, including systemic steroids (59%), chemotherapeutics (23%), immunomodulatory drugs (19.7%), antiepileptic drugs (8.2%), inhaled steroids (4.9%), intravenous immunoglobulin (1.6%), and antituberculosis drugs 1.6%.Bone pain was detected in 49.2%. All patients had vertebral fractures before treatment. Bisphosphonate treatment was given to 45 (73.8%). There was a significant increase in mean bone mineral density (BMD) and bone mineral content six months after treatment (both $p < 0.001$). There was a significant increase in BMD Z-score values for chronological and height age (both $p < 0.001$). Overall mean BMD values increased by

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Address for Correspondence: Emine Kübra Şen MD, Ankara University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey
E-mail: eminekubrasen@gmail.com **ORCID:** orcid.org/0000-0003-0633-2850

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31.15% with treatment. Following bisphosphonate treatment, there was a significant reduction in both fracture number and bone pain ($p < 0.01$). Similar benefits from bisphosphonate treatment were evident in those who did or did not receive steroid treatment.
Conclusion: Secondary osteoporosis is a condition that is influenced by many factors, such as the primary disease causing osteoporosis and chronic medication use, especially steroids. If left untreated, osteoporosis may lead to clinically important morbidity (bone pain, fractures, immobilization) and reduced linear growth of bone. When used to treat childhood secondary osteoporosis, bisphosphonates significantly improve BMD and reduced fracture risk.
Keywords: Childhood, secondary osteoporosis, bisphosphonate

Introduction

Osteoporosis is closely associated with a higher risk of fracture due to a reduction in bone mass and degradation of the microarchitecture of the bone (1). A significant proportion of maximum bone mass is achieved during childhood and adolescence. Childhood is thus a significant period for bone health and the development of a strong musculoskeletal system. To prevent long-term deformities, maintain bone health and improve quality of life, it is critical to identify, diagnose, treat, and manage individuals at risk of developing osteoporosis in this period (2).

When one or more vertebral fractures occur in children and adolescents without the presence of high-energy trauma, local disease, or a history of severe fractures with low bone density, it is considered osteoporosis (3). Osteoporosis can be divided into primary and secondary osteoporosis. Primary osteoporosis is caused by intrinsic skeletal problems, such as abnormalities in collagen, bone production, or bone mineralization. It can also be inherited. Otherwise, chronic drug usage or an underlying primary disease can result in secondary osteoporosis. Diseases leading to secondary osteoporosis in children can be grouped into neuromuscular diseases, endocrine disorders, metabolic diseases, chronic inflammatory diseases, and iatrogenic causes (4). Often, a clinical focus on the underlying primary disease and its treatment can lead to bone health being overlooked. Deterioration of bone health leads to fractures and immobilization and makes managing the patient more difficult. Some patients may be evaluated for osteoporosis only when a fracture develops (5).

In the treatment of osteoporosis, determination and treatment for the underlying causes are gaining importance. In terms of the osteoporosis, treatment aims are to reduce bone pain, increase bone mass, reduce the risk of fractures, and increase patient mobility. Bisphosphonates, synthetic pyrophosphate analogs that bind to osteoclasts, inhibiting function and promoting osteoclast apoptosis, are frequently used in treatment. There is limited information and studies on the dosage and duration of use of bisphosphonates in children, especially in secondary osteoporosis (6).

In this study, the aim was to determine the diagnostic findings in childhood secondary osteoporosis, to assess morbidities such as fractures and immobilization, to determine follow-up characteristics, and to investigate the efficacy of bisphosphonate treatments.

Methods

Patients diagnosed with secondary osteoporosis between January 2000 and January 2021 were included in the study. The study was retrospective, cross-sectional and descriptive. The protocol was approved by the Ethics Committee of Ankara University (approval number: İ7-441-20, date: 13.08.2020).

The underlying disease was defined as the "primary disease". Inclusion criteria were: aged <18 years at diagnosis of primary disease; currently being followed up in our clinic; the presence of one or more vertebral fractures in the absence of local disease or high-energy trauma; bone mineral density (BMD) Z-score ≤-2.0 on dual energy X-ray absorptiometry (DEXA) measurements; two or more long bone fractures by age ten years; three or more long bone fractures by age 19 years; and had a history of conditions that carry a risk of osteoporosis including chronic use of drugs known to affect bone metabolism, diseases that cause immobilization, or other diseases that may affect bone metabolism, such as some hormonal deficiencies or anorexia nervosa.

Exclusion criteria were: BMD Z-score above -2 and long bone fractures; BMD Z-score above -2 and no vertebral fractures; no history of chronic systemic disease and/or drug use; and those with a genetic or clinical diagnosis of primary osteoporosis.

Patient data were extracted from hospital patient files. Demographic data, underlying primary diseases and their characteristics, duration of immobilization, if any, chronic drug use, coexistence of additional endocrine diseases, presence and duration of bone pain, bone fracture characteristics and nutritional status were recorded. Patients were followed up regularly every six months. During physical examination, anthropometric characteristics and pubertal

stages of the patients were evaluated. In anthropometric evaluation, body weight, height, height standard deviation (SD) score (SDS), body mass index (BMI), and BMI% were recorded and evaluated according to national reference data (7).

Bone metabolism markers, including serum calcium (Ca), phosphorus (P), parathyroid hormone (PTH), 25-hydroxy vitamin D [25(OH)D], urinary Ca excretion, and additional biochemical measurements, if any, were analyzed in the pre-and post-treatment laboratory data of patients. Vitamin D deficiency was diagnosed when the serum 25(OH)D level was below 20 ng/mL (8).

DEXA (Hologic Explorer N/S91724, Software version 13.3.0.1) was used to measure lumbar (L1-4) vertebral BMD. BMD, bone mineral content (BMC), bone surface area, and Z-score were calculated according to normal reference values (9). Lateral vertebral radiographs were examined by pediatric radiologists and pediatric endocrinologists. Vertebral fractures were evaluated according to the Genant Classification (10). DEXA measurements were performed before treatment, at the sixth month of treatment and during the follow-up period at 6 month intervals. The last measurements of BMD were also recorded.

Characteristics of bisphosphonate treatment dose and duration were analyzed. Patients were treated with bisphosphonate therapy and pamidronate, zoledronic asid, or alendronate were all used during the study period. Different bisphosphonate options were chosen according to the current approaches to osteoporosis treatment, drug availability, and the patient's treatment preference after diagnosis of secondary osteoporosis over the 20 year study period. Pamidronate was administered at a dose of 1 mg/kg/day intravenously on three consecutive days and repeated every three months. Zoledronic acid was administered intravenously at a dose of 0.05 mg/kg every six months and alendronate was given orally at a dose of 70 mg/week. Ca supplementation was given for 14 days following treatment. Oral elemental Ca 25-50 mg/kg/day was given. The dose was adjusted according to blood Ca level. Patients with vitamin D deficiency were given vitamin D3 at a treatment dose of 2000 IU/day for at least six weeks. Maintenance treatment was given at a dose of 400-1000 IU/day depending on the blood 25(OH)D3 levels (8).

Statistical Analysis

Statistical Package for the Social Sciences, version 20 was used for statistical analysis (IBM Corp, Armonk, NY, USA). Descriptive statistics include mean, SD, median, minimum and maximum for continuous data, and count and percentage values are given for discrete data. The Shapiro-Wilk test was used to examine the conformity of the data to normal distribution. In comparing pre- and post-treatment laboratory values, BMD, BMI, and BMD Z-score values, the paired samples t-test was used for the data conforming to normal distribution and the Wilcoxon test was used for non-parametric data sets. The McNemar test was used for pre-treatment and post-treatment comparisons of nominal variables. Chi-square and Fisher's exact test were used for group comparisons of nominal variables (in cross-tabulation). A $p < 0.05$ was accepted as indicating statistical significance.

Results

The study included 61 patients (28 female; 45.9%). The most common underlying primary disease in patients with secondary osteoporosis was chronic inflammatory diseases (Table 1). The mean chronological age at the time of diagnosis of secondary osteoporosis was 11.89 ± 4.88 years and patients were evaluated for osteoporosis at a mean of 6.39 ± 5.13 years after the onset of underlying primary chronic diseases. At the time of diagnosis of secondary osteoporosis, the median height SDS (minimum-maximum height SDS) value was -1.64 (-9.60-1.80) and 23 (37.7%) had a height SDS below -2 SD and thus short stature. At the time of diagnosis, 54.1% were prepubertal (Table 2).

Chronic use of one or more drugs was present in 78.7%. These chronically taken drugs included systemic steroids (59%), chemotherapeutics (23%), immunomodulatory agents (19.7%), antiepileptic drugs (8.2%), inhaled steroids (4.9%), intravenous (iv) immunoglobulin (1.6%), and antituberculosis drugs (1.6%). Replacement therapies amongst patients included testosterone (1.6%), L-thyroxine (3.3%), estrogen (1.6%), and growth hormone (1.6%).

Bone pain was reported by 49.2%, and of these, 37.7% had low back pain. All patients had vertebral fractures before treatment (n = 61). Non-traumatic long bone fractures were present in 12.8% of the patients. The femur was

Table 1. Primary disease diagnoses of patients with secondary osteoporosis

	n	%
Chronic inflammatory disease	34	55.7
Neuromuscular disease	16	26.2
Immunodeficiency	8	13.1
Acute lymphoblastic leukemia	5	8.2
Metabolic disease	5	8.2
Solid organ transplantation	5	8.2
Bone marrow transplantation	4	6.6
Epilepsy	4	6.6

the most commonly fractured long bone (7.9%). Before endocrinological evaluation, the number of long bone fractures per year was 1 in 15.8% and 2 in 7.9% of cases.

The mean BMD of the cases included in the study was 0.47 ± 0.16 g/cm^2, and the BMD Z-score according to chronological age was -3.62 ± 1.16. Total BMC was 19.76 ± 10.51 g, and the mean BMD Z-score for height and age was -2.77 ± 1.63.

When the 25(OH)D3 levels of the cases were evaluated at the time of secondary osteoporosis diagnosis, the serum vitamin D level of 30 (49.1%) was below 20 ng/mL, and then vitamin D replacement was started. However, at the first six-month follow up after bisphosphonates, 17 (27.8%) had persistent serum vitamin D levels below 20 mg/mL despite vitamin D replacement.

Of the 61 patients, 45 (73%) received bisphosphonate treatment, and an additional two patients were referred to a different center for treatment. Sixteen patients who were diagnosed with secondary osteoporosis but did not attend follow-up or did not have regular follow-ups did not receive treatment. Patients received biphosphonates, such as zoledronic asid, pamidronate, or alendronate. The minimum duration of bisphosphonate treatment was 6 months and maximum 4 years (mean 1.2 years, median 1 year). Thirty-one patients (71%) received zoledronic acid treatment with the mean duration of 0.83 ± 0.45 years. Alendronate treatment was administered to five patients (11.6%) for an mean of 1.00 ± 0.61 years. Ten patients (23.3%) received pamidronate treatment with a mean duration of 1.65 ± 1.08 years. One patient received alendronate and then pamidronate. Furthermore, two patients received pamidronate and then zoledronic acid.

Table 2. Findings of patients diagnosed with secondary osteoporosis at the time of admission

	Mean ± SD	Median (min-max)
Age (years)	11.89 ± 4.88	11.6 (1.6-23)
Height SDS	-1.78 ± 2.19	-1.64 (-9.60-1.80)
BMI	17.91 ± 4.56	17 (11-28)
BMI%	94.24 ± 24.86	90 (61-180)
Pubertal status at admission	n	%
Prepubertal	33	54.1
Pubertal	28	45.9
Chronic drug use	n	%
1. Systemic steroid	48	78.7
2. Chemotherapetics	36	25
3. Immunomodulatory agent	14	23
4. Antiepileptic	12	19.7
5. Inhaled steroids	5	8.2
6. L-tyroxine	3	4.9
7. IVIG	2	3.3
8. Antituberculosis drugs	1	1.6
9. Testosterone	1	1.6
10. Estrogen	1	1.6
11. Growth hormone	1	1.6
Bone pain	29	49
Vertebral fractures	61	100

SD: standard deviation, BMI: body mass index, min-max: minimum-maximum, IVIG: intravenous immunoglobulin

With bisphosphonate treatment, there was an increase of 0.111 ± 0.09 g/cm^2 in the mean total BMD after six months of treatment ($p < 0.001$). Moreover BMD Z-scores increased six months after start of treatment ($p < 0.001$) (Figure 1). There was no difference between the mean area of L1-L4 vertebrae before and after treatment (Table 3). When the total BMD values of the patients before and after the last bisphosphonate treatment (mean treatment duration 1.10 ± 0.89 years) were compared, a mean increase of 0.169 ± 0.116 g/cm^2 was found ($p < 0.001$) with a mean increase of 31.15 ± 30.48% in the BMD values after six months of biphosphonate treatment compared to before treatment. However, there was no difference between the height SDS, BMI and BMI% of patients with secondary osteoporosis who received treatment before and six months after treatment. Pubertal progression was observed in eight patients. In only one case (2.3%), side effects were noted during bisphosphonate treatment. In this case, fever was recorded as an adverse effect approximately two hours after iv Zoledronic acid infusion.

There was a significant difference in reported bone pain and observed fracture rates before and after treatment with bisphosphanates ($p < 0.001$). Of the 27 patients with bone pain before treatment, the pain dissolved after treatment in 20 (74.1%). In 14 of 43 (32.5%) patients who had fractures before treatment, no fracture was detected after 6 months of treatment ($p < 0.05$). Only 1 of 32 (3.1%) patients without fracture had a non-traumatic fracture after treatment. Of note, no patients had long bone fractures after treatment.

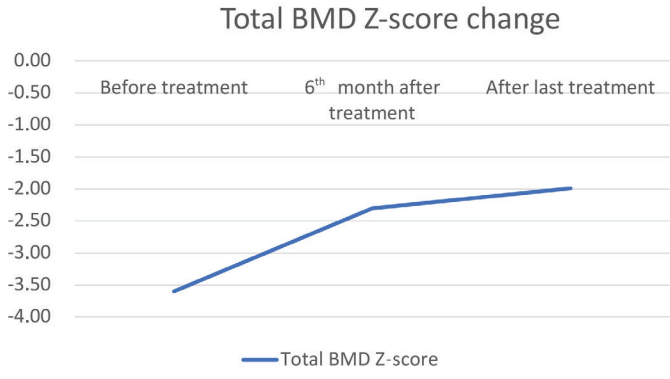

Figure 1. Median values of BMD Z-score of treated patients before treatment, six months after treatment and after the last treatment

BMD: bone mineral density

Thoracic and lumbar vertebral involvement significantly improved with treatment ($p < 0.01$). Of 26 patients with thoracic vertebrae pathology, improvement was seen in 11 (42.3%) patients with bisphosphonate treatment. Improvement in vertebral morphology (increase in vertebral BMD and height, reshaping of vertebral fractures) was seen in all cases with lumbar vertebral involvement ($n = 19$). There was no difference in the presence of scoliosis before and after treatment ($p > 0.05$). Serum Ca, P, alkaline phosphatase (ALP), vitamin D, and PTH levels did not differ between before and after biphosphonates ($p > 0.05$) (Table 3). Serum electrolytes were measured 4 hours, 24 hours and 1 week after the end of treatment. Hypocalcemia was not detected.

Cases with and without a history of steroids use were compared in terms of their response to bisphosphonate treatment (Table 4). There was an increase of 0.114 ± 0.086 g/cm^2 in the mean total BMD after the first six months of

Table 3. Clinical and laboratory values of patients diagnosed with secondary osteoporosis before bisphosphonate treatment, and at the sixth month of treatment

	Before treatment	Sixth month after treatment	Test statistics	p value
	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)		
Height SDS	-2.01 ± 2.28 -1.72 [(-9.60)-1.80]	-1.93 ± 2.11 -1.70 [(-8)-1.80]	Z = -0.369	0.712
BMI	17.47 ± 4.51 16 (11-28)	17.52 ± 4.59 17 (10-29)	Z = -0.557	0.577
BMI%	92.62 ± 21.97 90 (61-150)	92.88 ± 23.02 89 (58-150)	Z = -0.142	0.887
ALP (U/L)	204.35 ± 166.84 176.5 (26-978)	209.03 ± 178.40 183.5 (32-1004)	Z = -0.020	0.984
25(OH)D (µg/L)	25.74 ± 21.91 21.5 (3.6-118)	24.02 ± 13.57 23.5 (1-67)	Z = -0.077	0.939
PTH (pg/mL)	57.34 ± 68.80 36.5 (7-396)	53.14 ± 40.97 41.2 (6-202)	Z = -1.117	0.264
Total BMD (gr/cm^2)	0.422 ± 0.153 0.385 (0.165-0.830)	0.533 ± 0165 0.510 (0.298-0.884)	t = -7.157	< 0.001
Total BMC (gr)	16.90 ± 10.18 14.88 (0.33-42.30)	21.33 ± 10.50 19.00 (5.90-44.19)	Z = -4.766	< 0.001
L_1-L_4 area (cm^2)	35.36 ± 9.77 33.00 (17.95-60)	36.96 ± 9.51 37.27 (14.5-60)	t = -1.401	0.170
BMD Z-score	-3.88 ± 1.28 -3.60 [-6.80-(-0.96)]	-2.33 ± 1.56 -2.30 [-6.20-(-0.67)]	Z = -4.825	< 0.001
BMD Z-score by height	-3.19 ± 1.75 -3.12 [(-5.80)-3]	-1.55 ± 1.75 -1.76 [(-6.20)-3.30]	Z = -4.685	< 0.001

SDS: standard deviation score, BMI: body mass index, min-max: minimum-maximum, IVIG: intravenous immunoglobulin, BMD: bone mineral density, BMC: bone mineral content, ALP: alkaline phosphatase, PTH: parathyroid hormone, 25(OH)D: 25-hydroxy vitamin D, SD: standard deviation

Table 4. Comparison of the patients with history of chronic steroid use and those without before bisphosphonate treatment, at the sixth month of treatment, and at their last follow-up

	Patients using chronic steroids			Other patients		
	Before treatment	Sixth month after treatment	After latest treatment	Before treatment	Sixth month after treatment	After latest treatment
Age* (year)	11.1 ± 5.2	11.7 ± 5.3	11.9 ± 5.8	10.7 ± 4.4	11.2 ± 4.4	11.5 ± 4.6
Height SDS*	-1.74 ± 2.3	-1.72 ± 1.9	-1.70 ± 1.9	-2 ± 2.3	-2 ± 4.4	-1.3 ± 2.6
BMI*	17.6 ± 4.4	18.2 ± 4.4	18 ± 4.3	16.8 ± 4.1	18.3 ± 5.5	18.4 ± 5.2
BMI%*	95 ± 25	91.8 ± 22.2	92.9 ± 21.6	88.8 ± 24	97.4 ± 28.9	96.4 ± 24.3
Bone pain (%)	76	28	20	40	20	20
Bone fracture (%)	100	40	28	100	60	50
Total BMC* (g)	17.9 ± 9.5	21.8 ± 10.2	24.2 ± 11.3	15.8 ± 9.3	19.5 ± 10	21.2 ± 10
Total BMD* (g/cm^2)	0.422 ± 0.159	0.530 ± 0.163	0.592 **± 0.179**	0.394 ± 0.154	0.482 ± 0.154	0.536 ± 0.183
Total area* (cm^2)	36.4 ± 9.6	37.5 ± 10.2	38.4 ± 10.9	37 ± 9.6	37.1 ± 7.6	38.4 ± 8.5

*Mean ± SD for the analyzed parameters.
SDS: standard deviation score, BMI: body mass index, BMD: bone mineral density, BMC: bone mineral content

bisphosphonate treatment in patients with chronic steroid use ($p < 0.001$). An additional 0.05 ± 0.118 g/cm^2 increase in BMD ($p < 0.05$) was seen between this time point and the median last examination (1 year). Similar increases in BMD were seen in those not given steroid treatment (0.087 ± 0.052 g/cm^2 in first six months and 0.053 ± 0.086 g/cm^2 up to the last examination; $p < 0.001$). There was no significant difference ($p > 0.05$) between the percentage change in total BMD values of patients on or not on steroids but chronically taking other drugs (Table 5).

Discussion

In this study, the diagnostic features and response to bisphosphonate therapy was evaluated in a large group of children and adolescents with secondary osteoporosis over a twenty year period. The most common type of underlying primary disease in patients with secondary osteoporosis was chronic inflammatory diseases. Chronic medications, most frequently steroids, were used in most of the patients.

The mean age of patients diagnosed with osteoporosis was 11.89 years. Similar ages were reported in the literature. In a study by Inoue et al. (11) 39 patients with secondary osteoporosis were analyzed and the mean age at diagnosis of secondary osteoporosis was 12 years. Zacharin et al. (12) reported that the mean age at diagnosis of secondary osteoporosis was 10.1 years in their series of patients with Duchenne muscular dystrophy.

Almost one-third of our cases had short stature at the time of diagnosis. The duration and severity of chronic systemic diseases and the use of drugs that affect growth, especially steroids, are factors that are known to cause growth retardation. DEXA measurements in bone give areal measurement only and not volumetric values. DEXA measures a three-dimensional object in two dimensions spatially, and thus bone size affects the measurement result. So, they may give lower measurement results in short children than in children of normal height of the same age (13). Thus, we would like to stress that short stature should be assessed in cases with chronic disease and that evaluations should be adjusted accordingly.

Since the primary diseases examined in the present study and in earlier reports differ between centers, the age at diagnosis and the duration of development of osteoporosis after the primary disease may differ (14,15). Variables affecting this include the place/country where the centers are located, population differences and the clinical experience of the center (16,17). In our cohort, cases with secondary osteoporosis were referred to our pediatric endocrinology department fairly late after the diagnosis of the primary disease (6.39 ± 5.13 years). This suggests that there may be little awareness that bone health can be affected during various primary diseases, and secondary osteoporosis may be diagnosed late. Moreover, that the mean age of first fracture was 6.63 ± 4.31 years actually emphasizes the delay in diagnosing osteoporosis despite the early fracture. Evaluation of osteoporosis in patients with chronic diseases before fractures develop is important in terms of minimizing morbidity. Focusing only on long bone fractures may delay diagnosis of secondary osteoporosis. In chronically ill patients who are at risk of secondary osteoporosis, risk factors should be investigated and lateral vertebral radiography may be justified, even in the absence of long bone fracture. We recommend that clinicians who follow chronically ill pediatric patients should evaluate them for osteoporosis with lateral vertebral radiography.

The most common type of primary disease was chronic inflammatory diseases, followed by neuromuscular diseases. Factors such as increased osteoclastic activity as a result of increased cytokines in chronic inflammatory diseases, disruption of the mechanostat mechanism in neuromuscular diseases, increased immobilization, steroids used in their treatment, anticonvulsant treatment and malnutrition may lead to the development of secondary osteoporosis (1,12). Clinicians should not neglect careful evaluation in terms of bone health, especially in the follow-up of this group of diseases. In addition to the primary disease, several drugs may also play a role in the development of osteoporosis. Most of the patients were on one or more chronic medications. Long-term steroid use is normal in the management of these diseases and 63.9% of our cohort had a history of systemic and/or inhaled steroid use. However, the characteristics of the cases with secondary osteoporosis

Table 5. Comparison of percent change in total BMD between steroid-treated patients and other patients

	Steroid	Other	Test statistics	p value
	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)		
Total BMD percentage change	19.77 ± 9.95 20.14 (6.49-39.33)	37.09 ± 35.76 27.27 (-9.80-111.51)	U = 109.0	0.327

SD: standard deviation, min-max: minimum-maximum, BMD: bone mineral density

in the present study and in the literature may differ as well as exhibiting similarities. This is because factors, such as the heterogeneity of the patients' primary diseases, the demographics of study populations, the nature of the drugs used, and the different duration of drug use affect the results of the studies (18,19).

Vitamin D deficiency was detected in very nearly half of our cohort. Although replacement was given in cases with deficiency, vitamin D was still low in more than half (17/30) of these cases at the end of the six months follow-up. Optimal levels of vitamin D are crucial for maintaining bone health (20). Focusing on underlying problems may lead to neglect of checking vitamin D levels and thus failing to treat the deficiency, if any. Care should be taken to bring vitamin D levels to normal limits in chronically ill patients, to avoid having an avoidable negative effect on bone health. In patients with secondary osteoporosis, treatment of the etiology, if possible, will also prevent the negative effect on bone metabolism. If etiologic factors persist, it would be appropriate to evaluate bone health, eliminate vitamin D deficiency, and continue long-term follow-up of patients with osteoporosis, with appropriate management.

There is no consensus or treatment guideline for the treatment of secondary osteoporosis in children. In the present study, bisphosphonate treatment was administered to 45 patients over a period of 20 years in our clinic. Galindo-Zavala et al. (21) recommend zoledronic acid, alendronate and pamidronate for the treatment of secondary osteoporosis in children. Simm et al. (22) in 2018, treatment with either 0.1 mg/kg/year iv zoledronic acid or 9 mg/kg/year iv pamidronate were recommended for the treatment of primary and secondary osteoporosis in children. One year after treatment, the patient should be evaluated. If bone pain and/or bone fracture are present after this evaluation, the BMD Z-score is <-2, and immobilization or steroid use continues, bisphosphonate treatment should be continued for another year. In the present study, bone pain and non-traumatic fracture frequency of patients decreased significantly after treatment. The mean total BMD and BMD Z-score significantly increased by DEXA assessment after bisphosphonate treatment. Celin et al. (23) analyzed 24 studies investigating the effect of bisphosphonate treatment on bone pain and fracture frequency. These authors reported that bisphosphonates were used to relieve bone pain caused by a wide variety of causes. Twenty of twenty-four studies found a benefit of bisphosphonates in relieving bone pain due to different pathologies. A notable decrease in bone pain was observed following treatment in research by Al-Agha et al. (24) investigating the safety and effectiveness of zoledronic acid therapy in the treatment of secondary osteoporosis. In a study by Sees et al. (25) in children with cerebral palsy, the frequency of fractures after pamidronate treatment for osteoporosis was evaluated. It was reported that a significant decrease in the fracture rate was detected after treatment, although the most commonly fractured bone before and after treatment was the femur. In the study by Allington et al. (26) in which cyclic pamidronate treatment was evaluated in secondary osteoporosis, including cerebral palsy and other neuromuscular diseases, a significant difference was found between the total BMD Z-scores of 18 patients examined before and 1 year after treatment. Naithani et al. (27) found a significant increase in total BMD Z-score values before and after Zoledronic acid treatment in 27 patients with osteoporosis secondary to beta-thalassemia. Lee et al. (28) showed a significant difference between pre-treatment and post-treatment total BMD Z-scores with pamidronate treatment of osteoporosis secondary to chemoterapy in acute lymphoblastic leukemia and non-Hodgkin lymphoma. It has been shown that bisphosphonates are beneficial in the treatment of secondary osteoporosis and are an effective treatment in reducing bone pain and bone fractures, and this finding is supported by our study.

It is well known that steroids have negative effects on bone metabolism and cause osteoporosis. In the present study, when the total BMD parameters of patients using steroids and other patients before and six months after treatment were compared, it was found that both groups were similarly affected at the time of osteoporosis diagnosis. There are no published studies in which the pre-treatment and post-treatment characteristics of secondary osteoporosis patient groups with and without chronic steroid use were compared. However, studies examining the treatment of patients with osteoporosis secondary to chronic steroid use have been reported. In a group of pediatric patients with nephrotic syndrome who developed osteoporosis secondary to chronic steroid use, a significant increase was found in total BMD values at the third month after pamidronate treatment (29). Ward et al. (30) investigated zoledronic acid treatment in 18 patients who developed osteoporosis secondary to steroids and a significant increase was found in the total BMD of the patients at the twelfth month after drug administration.

In the present study, only 1 (2.3%) patient receiving bisphosphonate treatment had side effects. The patient's clinical appearance, physical examination findings, and fever did not suggest an infectious condition. Furthermore, there was no significant difference between pre- and post-treatment Ca, P, ALP, vitamin D and PTH values. In the study by Ooi et al. (31) no significant difference was found between serum Ca, P, ALP, and spot urine Ca/creatinine

ratio before and 18 months after treatment, and serum bone metabolism biomarkers before and after treatment were found to be within normal limits, as in our study. In a study by Munns et al. (32), hypocalcemia developed in 74% of patients, fever in 52%, nausea/vomiting in 35%, and headache in 17% after zoledronic acid infusion in 63 patients with osteoporosis. Nosomyant et al. (33) reported that flu-like symptoms developed in 7% of patients and hypocalcemia developed in 7% of patients after iv infusion of zoledronic acid and pamidronate in 123 patients diagnosed with osteoporosis. In another study by Högler et al. (34) it was reported that influenza-like symptoms were found in 85% of patients following iv zoledronic acid infusion. One of the possible reasons for the low rate of side effects in our cohort may be that the side effects of iv bisphosphonate treatment may have been either less critical and or not recorded because of their primary diseases.

There is no clear recommendation on how long bisphosphonates should be used in the treatment of osteoporosis in children. Side effects that may occur in long-term use of bisphosphonates are not yet known. In the pediatric age group, bone tissue is a growing tissue, and the hormonal status is different from that of adults. Considering these differences, the results of long-term use of bisphosphonates in children may be different from those in adults.

Study Limitations

The limitation of our study was the relatively short follow-up period in our patients treated for osteoporosis and the long term of follow-up of these patients is planned. Another limitation of our study is the use of different treatment protocols in the treatment of secondary osteoporosis during the long study period.The strengths of our study are that it was a large series of secondary osteoporosis in childhood diagnosed in a single center, that the data of patients with and without a history of chronic steroid use was compared, and that follow-up data at six months after treatment are given.

Conclusion

In conclusion, pediatric secondary osteoporosis is a condition that is influenced by many factors, such as the primary disease causing osteoporosis, and chronic medication use, especially steroids. This study also highlights that in children with chronic diseases, clinicians should evaluate the patient for osteoporosis based on risk factors. If left untreated, osteoporosis may lead to essential diseases, such as bone pain, bone fractures, immobilization and reduced linear growth of bone. It is important that early recognition of secondary osteoporosis is made and optimal care with vitamin D and Ca intake should be provided. It has been shown that bisphosphonates are an effective treatment modality in treating childhood secondary osteoporosis and reducing the incidence of fractures resulting from osteoporosis and these findings are supported by the results of the present study.

Ethics

Ethics Committee Approval: The protocol was approved by the Ethics Committee of Ankara University (approval number: İ7-441-20, date: 13.08.2020).

Informed Consent: The study was retrospective, cross-sectional and descriptive.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Emine Kübra Şen, Merih Berberoğlu, Gizem Şenyazar, Sirmen Kızılcan Çetin, Elif Özsu, Zehra Aycan, Zeynep Şıklar, Concept: Emine Kübra Şen, Merih Berberoğlu, Zeynep Şıklar, Design: Emine Kübra Şen, Merih Berberoğlu, Zeynep Şıklar, Data Collection or Processing: Emine Kübra Şen, Gizem Şenyazar, Sirmen Kızılcan Çetin, Ayşegül Ceran, Seda Erişen Karaca, Analysis or Interpretation: Emine Kübra Şen, Literature Search: Emine Kübra Şen, Zeynep Şıklar, Writing: Emine Kübra Şen, Merih Berberoğlu, Gizem Şenyazar, Sirmen Kızılcan Çetin, Elif Özsu, Zehra Aycan, Zeynep Şıklar.

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References

1. Shaw NJ. Management of osteoporosis in children. Eur J Endocrinol. 2008;159(Suppl 1):S33-39. Epub 2008 Sep 4.
2. Madhuchani D, Seneviratne SN, Ward LM. Bone health in childhood and adolescence: an overview on dual-energy X-ray absorptiometry scanning, fracture surveillance and bisphosphonate therapy for low-middle-income countries. Front Endocrinol (Lausanne). 2023;14:1082413.
3. Shuhart CR, Yeap SS, Anderson PA, Jankowski LG, Lewiecki EM, Morse LR, Rosen HN, Weber DR, Zemel BS, Shepherd JA. Executive Summary of the 2019 ISCD Position Development Conference on Monitoring Treatment, DXA Cross-calibration and Least Significant Change, Spinal Cord Injury, Peri-prosthetic and Orthopedic Bone Health, Transgender Medicine, and Pediatrics. J Clin Densitom. 2019;22:453-471. Epub 2019 Jul 5
4. Özen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4:46-56. Epub 2016 Dec 30
5. Dittrich AT, Janssen EJ, Geelen J, Bouman K, Ward LM, Draaisma JM. Diagnosis, Follow-Up and Therapy for Secondary Osteoporosis in Vulnerable Children: A Narrative Review. Appl Sci. 2023;13:4491.

6. Jin HY, Noh ES, Hwang IT. Diagnosis and Management of Osteoporosis in Children and Adolescents. Ewha Med J. 2023;46:e18.
7. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.
8. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-1930. Epub 2011 Jun 6 Erratum in: J Clin Endocrinol Metab. 2024;109:e1991.
9. Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A Comprehensive Online Calculator for Pediatric Endocrinologists: ÇEDD Çözüm/TPEDS Metrics. J Clin Res Pediatr Endocrinol. 2017;9:182-184. Epub 2017 Apr 26
10. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993;8:1137-1148.
11. Inoue Y, Mitsunaga K, Yamamoto T, Chiba K, Yamaide F, Nakano T, Morita Y, Yamaide A, Suzuki S, Arima T, Yamaguchi KI, Tomiita M, Shimojo N, Kohno Y. Early use of alendronate as a protective factor against the development of glucocorticoid-induced bone loss in childhood-onset rheumatic diseases: a cross-sectional study. Pediatr Rheumatol Online J. 2018;16:36.
12. Zacharin M, Lim A, Gryllakis J, Siafarikas A, Jefferies C, Briody J, Heather N, Pitkin J, Emmanuel J, Lee KJ, Wang X, Simm PJ, Munns CF. Randomized Controlled Trial Evaluating the Use of Zoledronic Acid in Duchenne Muscular Dystrophy. J Clin Endocrinol Metab. 2021;106:2328-2342.
13. Lim DBN, Moon RJ, Davies JH. Advances in Diagnosis and Management of Childhood Osteoporosis. J Clin Res Pediatr Endocrinol. 2022;14:370-384. Epub 2022 Aug 19
14. Saraff V, Högler W. Endocrinology and adolescence: osteoporosis in children: diagnosis and management. Eur J Endocrinol. 2015;173:185-197. Epub 2015 Jun 3
15. Golden NH, Abrams SA; Committee on Nutrition. Optimizing bone health in children and adolescents. Pediatrics. 2014;134:e1229-1243.
16. Steelman J, Zeitler P. Treatment of symptomatic pediatric osteoporosis with cyclic single-day intravenous pamidronate infusions. J Pediatr. 2003;142:417-423.
17. Ooi HL, Briody J, Biggin A, Cowell CT, Munns CF. Intravenous zoledronic Acid given every 6 months in childhood osteoporosis. Horm Res Paediatr. 2013;80:179-184. Epub 2013 Sep 18
18. Lim A, Simm PJ, James S, Lee SL, Zacharin M. Outcomes of Zoledronic Acid Use in Paediatric Conditions. Horm Res Paediatr. 2021;93:442-452. Epub 2021 Jan 28
19. George S, Weber DR, Kaplan P, Hummel K, Monk HM, Levine MA. Short-Term Safety of Zoledronic Acid in Young Patients With Bone Disorders: An Extensive Institutional Experience. J Clin Endocrinol Metab. 2015;100:4163-4171. Epub 2015 Aug 26
20. Winzenberg T, Jones G. Vitamin D and bone health in childhood and adolescence. Calcif Tissue Int. 2013;92:140-150. Epub 2012 Jun 19
21. Galindo-Zavala R, Bou-Torrent R, Magallares-López B, Mir-Perelló C, Palmou-Fontana N, Sevilla-Pérez B, Medrano-San Ildefonso M, González-Fernández MI, Román-Pascual A, Alcañiz-Rodríguez P, Nieto-Gonzalez JC, López-Corbeto M, Graña-Gil J. Expert panel consensus recommendations for diagnosis and treatment of secondary osteoporosis in children. Pediatr Rheumatol Online J. 2020;18:20.
22. Simm PJ, Biggin A, Zacharin MR, Rodda CP, Tham E, Siafarikas A, Jefferies C, Hofman PL, Jensen DE, Woodhead H, Brown J, Wheeler BJ, Brookes D, Lafferty A, Munns CF; APEG Bone Mineral Working Group. Consensus guidelines on the use of bisphosphonate therapy in children and adolescents. J Paediatr Child Health. 2018;54:223-233.
23. Celin MR, Simon JC, Krzak JJ, Fial AV, Kruger KM, Smith PA, Harris GF. Do Bisphosphonates Alleviate Pain in Children? A Systematic Review. Curr Osteoporos Rep. 2020;18:486-504. Erratum in: Curr Osteoporos Rep. 2022;20:226.
24. Al-Agha AE, Shaikhain TA, Ashour AA. Safety & Efficacy of Cyclic Zoledronic Acid Therapy on Pediatric Secondary Osteoporosis. Glob J Health Sci. 2016;8:48648.
25. Sees JP, Sitoula P, Dabney K, Holmes L Jr, Rogers KJ, Kecskemethy HH, Bachrach S, Miller F. Pamidronate Treatment to Prevent Reoccurring Fractures in Children With Cerebral Palsy. J Pediatr Orthop. 2016;36:193-197.
26. Allington N, Vivegnis D, Gerard P. Cyclic administration of pamidronate to treat osteoporosis in children with cerebral palsy or a neuromuscular disorder: a clinical study. Acta Orthop Belg. 2005;71:91-97.
27. Naithani R, Seth T, Tandon N, Chandra J, Choudhry VP, Pati H, Saxena R. Zoledronic Acid for Treatment of Low Bone Mineral Density in Patients with Beta Thalassemia Major. Indian J Hematol Blood Transfus. 2018;34:648-652. Epub 2018 Mar 27
28. Lee JM, Kim JE, Bae SH, Hah JO. Efficacy of pamidronate in children with low bone mineral density during and after chemotherapy for acute lymphoblastic leukemia and non-Hodgkin lymphoma. Blood Res. 2013;48:99-106. Epub 2013 Jun 25
29. Kim SD, Cho BS. Pamidronate therapy for preventing steroid-induced osteoporosis in children with nephropathy. Nephron Clin Pract. 2006;102:81-87. Epub 2005 Nov 10
30. Ward LM, Choudhury A, Alos N, Cabral DA, Rodd C, Sbrocchi AM, Taback S, Padidela R, Shaw NJ, Hosszu E, Kostik M, Alexeeva E, Thandrayen K, Shenouda N, Jaremko JL, Sunkara G, Sayyed S, Aftring RP, Munns CF. Zoledronic Acid vs Placebo in Pediatric Glucocorticoid-induced Osteoporosis: A Randomized, Double-blind, Phase 3 Trial. J Clin Endocrinol Metab. 2021;106:5222-5235.
31. Ooi HL, Briody J, McQuade M, Munns CF. Zoledronic acid improves bone mineral density in pediatric spinal cord injury. J Bone Miner Res. 2012;27:1536-1540.
32. Munns CF, Rajab MH, Hong J, Briody J, Högler W, McQuade M, Little DG, Cowell CT. Acute phase response and mineral status following low dose intravenous zoledronic acid in children. Bone. 2007;41:366-370. Epub 2007 May 13
33. Nasomyont N, Hornung LN, Gordon CM, Wasserman H. Outcomes following intravenous bisphosphonate infusion in pediatric patients: A 7-year retrospective chart review. Bone. 2019;121:60-67. Epub 2019 Jan 4
34. Högler W, Yap F, Little D, Ambler G, McQuade M, Cowell CT. Short-term safety assessment in the use of intravenous zoledronic acid in children. J Pediatr. 2004;145:701-704.

CASE REPORT

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Long-term Follow-up of a Late Diagnosed Patient with Temple Syndrome

Nikolinka Yordanova[1], Violeta Iotova[1], Deborah J. G. Mackay[2,3], I. Karen Temple[3], Sara Stoyanova[1], Mari Hachmeriyan[4]

[1]Medical University-Varna, Department of Pediatrics, Varna, Bulgaria
[2]Wessex Regional Genetics Laboratory, Salisbury Foundation NHS Trust, Salisbury, United Kingdom
[3]University of Southampton Faculty of Medicine, Department of Medical Genetics, Southampton, United Kingdom
[4]Medical University, Department of Medical Genetics, Varna, Bulgaria

What is already known on this topic?

The presented patient's history and disease course over more than 18 years are consistent with other reported Temple syndrome patients in the literature, regardless of the late diagnosis and childhood follow-up as a suspected other conditions.

What this study adds?

The description of the case shows the significance of multidisciplinary life-long follow-up for the patients with rare endocrine disease. Our patient is the only one genetically confirmed in Bulgaria and the second in the world with signs of clinical and biochemical hyperandrogenism. This is an intriguing finding that deserves future studies. The article is significant because it follows the trend for developing and expansion of the Rare Endocrine Networks all over the world in order to provide specialized multidisciplinary care for the rare patients.

Abstract

Temple syndrome is a rare imprinting disorder, caused by alterations in the critical imprinted region 14q32 of chromosome 14. It is characterized by pre- and postnatal growth retardation, truncal hypotonia and facial dysmorphism in the neonatal period. We report an 18-year-old girl with a late diagnosis of Temple syndrome presenting with all typical signs and symptoms including small for gestational age at birth, feeding difficulties, muscle hypotonia and delayed developmental milestones, central precocious puberty, truncal obesity and reduced growth. The patient is the second reported in the literature with signs of clinical and biochemical hyperandrogenism and the first treated with Dehydrocortisone®, with a good response. The clinical diagnosis of this patient was made after long-term follow up at a single center for rare endocrine diseases, and a molecular genetics diagnosis of complete hypomethylation of 14q32 chromosome imprinting center (DLK/GTL2) was recently established. Growth hormone treatment was not given and although precocious puberty was treated in line with standard protocols, her final height remained below the target range. Increased awareness of Temple syndrome and timely molecular diagnosis enables improvement of clinical care of these patients as well as prevention of inherent metabolic consequences.

Keywords: Temple syndrome, late diagnosis, long-term follow-up

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Address for Correspondence: Nikolinka Yordanova MD, Medical University-Varna, Department of Pediatrics, Varna, Bulgaria
E-mail: nikolinka.yordanova@mu-varna.bg; nikolinkayordanova@yahoo.com
ORCID: orcid.org/0000-0003-2806-026X

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Introduction

We present a case report and long-term follow-up of a patient with a late diagnosis of Temple syndrome, to raise awareness among clinicians of the importance of timely diagnosis of this disease. Although some of the patient's conditions arose during her childhood, and she was treated in line with standard protocols, her final outcomes are poorer than she might have achieved if her diagnosis had been established at an earlier age. The report also highlights novel endocrine findings.

Temple syndrome was first described by Temple et al. (1) in 1991, who reported a male, aged 18 years, who inherited a balanced Robertsonian translocation from his mother and as a result had maternal uniparental disomy of chromosome 14 (mUPD14). Gillessen-Kaesbach et al. (2) published eight patients with clinical features of mUPD14 in 2008, expanding the phenotype.

Imprinting defects in Temple syndrome lead to incorrect expression of imprinted genes on chromosome 14q32. Maternally expressed genes in 14q32, *MEG3*, *RTL1as* and *MEG8*, as well as the paternally expressed genes, *DLK1* and *RTL1*, are regulated by two differentially methylated regions (DMR), both methylated on the paternal and unmethylated on the maternal copy (3). Temple et al. (4) reported a patient with aberrant loss of paternal methylation at the 14q32 IG-DMR. A year later, Buiting et al. (5) described three patients with similar genetic characteristics who showed loss of methylation of the paternal copy of the *DLK1-GTL2-DIO3* domain. It is now established that mUPD14, loss of methylation and rare paternal deletions of the locus can cause Temple syndrome (6,7).

Intrauterine growth retardation, low birth weight, early neonatal muscular hypotonia, delayed early motor milestones and feeding problems are the clinical hallmarks of Temple syndrome (ORPHA:254516, OMIM#616222) (1,2). Postnatal clinical course is further characterised by persisting growth retardation, subtle facial dysmorphism (broad forehead and short nose with a wide nasal tip), joint hypermobility, small hands and feet, precocious puberty, truncal obesity and short stature at adulthood (1,8,9,10). Speech delay can be present in infancy to early childhood, but verbal capacity usually normalizes in childhood. Some patients have intellectual delay, and autism has been reported (11,12,13). Patients are prone to late metabolic complications, particularly obesity (8,10).

Informed consent for this publication was obtained from the patient and the family.

Case Report

We report an 18-year-old girl with Temple syndrome. Our patient is the first child of non-consanguineous parents, with a family history of short stature (the paternal grandmother) without other clinical associations.

The singleton pregnancy was uneventful. Delivery was natural at 38th gestational week, with meconium-stained amniotic fluid. Resuscitation was required initially. The girl was small for gestational age (SGA) with a birth weight at the 2nd percentile (2350 g), birth length at the 25th percentile (48 cm) and head circumference at the 10th percentile (34 cm), (https://www.cdc.gov/growthcharts/clinical_charts.htm). Truncal hypotonia, poor growth and lack of weight gain were noticed in the neonatal and early infant period. Nasogastric tube feeding was introduced because of feeding difficulties until three months of age.

At the age of 7 months, Silver-Russell syndrome (SRS) was suspected (Table 1), but genetic testing for maternal UPD 7 and methylation at H19 gave negative results.

The girl demonstrated motor and speech delay. Her first steps were at 18 months and first words at the age of two years, with the help of a speech therapist. At the age of 14 months she had an episode of severe hypoglycaemia with generalized seizure. The inability to endure long periods of fasting remained until pre-school age. The family was educated to recognize, measure and cope with hypoglycaemia at home.

Between birth and six years of age, the patient's height was below the 3rd percentile on the Centers for Disease Control and Prevention appropriate for age and sex growth chart. After four years of age she started to gain weight and moved from the 25th to the 75th weight percentile with no improvement in linear growth (Figure 1). At the age of 6 years and 10 months, because of a further decrease of growth velocity, recombinant human growth hormone (rhGH) treatment was prescribed under the approved indication of being born SGA without postnatal catch-up. During the process of supplying the family with rhGH, the patient presented with signs of precocious puberty. Over three months, at the age of 7 years 2 months, she developed thelarche, pubic hair, and increased growth velocity. Her bone age accelerated to 9.5 years by the Greulich and Pyle method. Due to the rapidity of pubertal progression luteinizing hormone releasing hormone (LHRH) agonist treatment (triptorelin 3.75 mg i.m. every 28 days) was started at the age of 7 years 5 months, with good compliance. Of note, rhGH was never used.

The girl was treated until 11 years of age without further pubertal progression and with decreased growth velocity,

but with continuing rapid weight gain. The discrepancy between her short stature and progressive truncal obesity increased with time (Figure 1). Prader-Willi syndrome (PWS) was suspected at 10 years of age because of the clinical overlap in the neonatal period, some of the dysmorphic features and most of all, the uncontrollable weight gain (Figure 2). However, the methylation test gave a negative result for PWS. Facial acne appeared for the first time at the age of nine years.

After LHRH agonist therapy was withdrawn, rapid pubertal progression followed, with menarche at 12 years 4 months. Elevated 17-hydroxyprogesterone (17-OHP), testosterone, and androstendione were detected at the age of 16 years 10 months in parallel with worsening of acne, complaints of oily hair and mild hirsutism (Ferriman-Gallwey score of 13 out of 36 points). Prednisone was started at a daily dose of 5 mg prior to night sleep for six months. An attempt was made to reduce prednisone to 2.5 mg/d thereafter but because of a worsening of hyperandrogenism the dose was again increased to 5.0 mg/d (Table 2).

The accumulating features and events during the whole 18-year patient follow-up led to a clinical diagnosis of Temple syndrome, which was molecularly confirmed at the Wessex Regional Genetic Laboratory, United Kingdom, after written informed consent from the patient and the family. The patient's DNA showed complete hypomethylation of the *DLK1/GLT2* imprinting centre on chromosome 14q32, consistent with the diagnosis of Temple syndrome.

After diet and physical activity counseling throughout childhood and adolescence, the patient lost weight and is now controlling her weight successfully. Her adult height is 141 cm [-4.76 standard deviation score (SDS)], 5 cm below the lower limit of the target range, with current BMI of 23.6 kg/m^2 (0.48 SDS) (Figure 1). The patient's metabolic markers (blood glucose level, insulin level, homeostasis model assessment-estimated-insulin resistance, lipids) are all within reference range. Facial acne improved with time and treatment. Clinical and biochemical hyperandrogenism abated and gradual improvement in menstrual regularity followed. She is seen every six months because of the increased risk of metabolic complications. Transition to adult endocrinologists at the same rare endocrine diseases expert center is ongoing.

Discussion

The presented girl is the first described and confirmed Bulgarian patient with Temple syndrome. Her history demonstrates the natural history of this condition after

Table 1. Differential diagnosis - clinical overlap with Silver-Russell and Prader-Willi syndrome, based on Ioannides et al. (8), Kagami et al. (10), Wakeling et al. (23), Goldstone et al. (24)

Clinical feature	Our patient	Silver-Russell syndrome (24)	Prader-Willi syndrome (23)	Features of Temple syndrome, Kagami et al. (10) (n = 32)	Features of Temple syndrome, Ioannides et al. (8) (n = 51)
Intrauterine growth retardation	+	+	Mild	84 %	75 %
Postnatal growth retardation	+	+	+	94 %	79 %
Delayed early motor milestones	+	+	+	*	83 %
Feeding problems	+	+	+	63 %	*
Early neonatal hypotonia	+	-	+	68 %	93 %
Broad forehead	+	+	-	63 %	*
Small feet	+	-	+	91 %	96 %
Obesity	+	+	+	11 %	49 %
Precocious puberty	+	+	Rarely	76 %	86 %
Short stature at adulthood	+	+	+	*	*
Diabetes mellitus	-	+	+	11 %	*

*: no data

Table 2. Androgen levels and treatment doses of prednisone during follow-up

	16 y 10 mo	17 y 1 mo	17 y 7 mo
17-hydroxyprogesterone, ng/mL	**9.78** (0.2-1.3)	1.2 (1-4.5)	**1.59** (0.2-1.3)
Androstendione, nmol/L (1-11.5)	**13.4**	6.3	11.5
Testosterone, nmol/L (0-1.38)	**2.13**	-	-
Prednisone	**5 mg**	**2.5 mg**	**5 mg**

y: year, mo: month

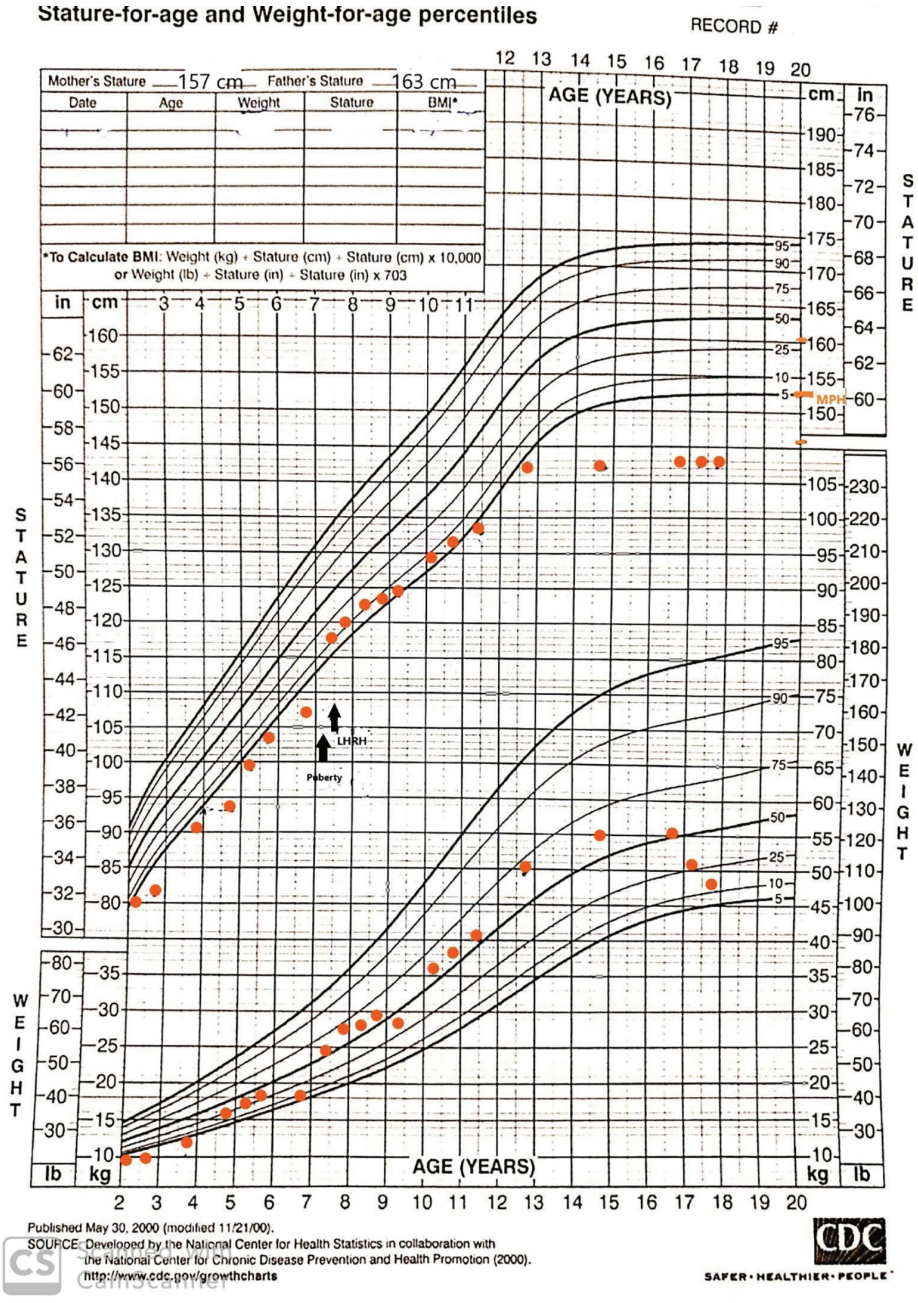

Figure 1. Growth chart (https://www.cdc.gov/growthcharts/clinical_charts.htm)

careful follow-up over 18 years in a single center, and provides insights into the complex growth pattern that is observed in the absence of rhGH treatment. To our knowledge, there are only two other reports of patients with late diagnosis of Temple syndrome, detailing long term follow up of 13 years and at 33 years (6,13). Previous testing for two imprinting conditions (SRS and PWS) adds to the literature showing the clinical similarity between these conditions and patients with Temple syndrome, and showing that multi-locus imprinting investigation is warranted if an imprinting disorder is suspected (14).

Clinical features of Temple syndrome are heterogeneous and age dependent (15,16). Patients often present with some of the features of SRS (17). Kagami et al. (10) showed that SRS-like phenotype was present in 20% of patients with Temple syndrome, differential diagnosis being particularly difficult in infancy (11,18). Genetic confirmation of Temple syndrome has been reported among patients previously tested for PWS (15,19).

Evidence clearly indicates that Temple syndrome is more prevalent than previously recognized (8,10,16). When

Figure 2. The patient self-taken photograph at 10 years of age

clinical findings for SRS and PWS are observed and there is no genetic confirmation, Temple syndrome should be the next suspected condition.

The final diagnosis, established at the age of 18 years in the presented patient, is an important achievement for her. Although there is currently no causal treatment for Temple syndrome, concomitant features and especially metabolic complications can be prevented or treated (13). A failure of early diagnosis prevented the patient from accessing rhGH treatment under the SGA indication for children without postnatal catch-up. However, not all patients with Temple syndrome are eligible for rhGH under this indication, because their early growth parameters may fall within low normal ranges (20). Children who were treated with a median rhGH dose of 0.040 mg/kg/day, had a median 1.31 SDS increase in height for the first year and increased height velocity of 5.30 cm/year. They had similar short-term response to rhGH as other treated SGA patients (21). An established diagnosis of Temple syndrome could facilitate the treatment process, and would have led to rhGH treatment of the current patient regardless of the difficulties in the supplies at that time. The missed opportunity for therapy with rhGH is one of the shortcomings in the patient's management.

As mentioned above, at the age of 16 years the patient presented with signs of hyperandrogenism. To the best of our knowledge, there is only one published report of a patient with Temple syndrome with isolated hypomethylation of the 14q32 imprinted *DLK1/MEG3* region who had clinical signs of hyperandrogenism (6). Our patient's results indicated markedly elevated basal 17-OHP. Although ACTH stimulation test was not done, the findings were consistent with non-classical congenital adrenal hyperplasia (NCAH). According to Nördenstrom and Falhammar, basal 17-OHP of above 15 nmol/L (4.7 ng/mL) and/or ACTH-stimulated 17-OHP of more than 30 nmol/L (9.43 ng/mL), in females during the follicular phase of the menstrual cycle, is considered to be diagnostic for NCAH (22). For that reason, the clinical diagnosis of NCAH was established in our patient and therapy with prednisone was prescribed, with good response nine months after the start of treatment (Table 2). Further genetic testing may be warranted to exclude NCAH. Hyperandrogenism may also be a metabolic consequence of Temple syndrome that is not yet investigated in patients of the appropriate age.

Patients with Temple syndrome may develop obesity, type 2 diabetes mellitus, hypercholesterolemia/hyperlipidemia, and obstructive sleep apnea (12,13). To date, the patient has not shown any of these features, most likely because of her current successful weight control.

Conclusion

The clinical history of this patient over more than 18 years of follow up is consistent with other reports of patients with Temple syndrome who received late diagnosis after earlier clinical investigation for SRS and PWS. The observation of adrenal hyperandrogenism is an intriguing finding that deserves future study and further investigation. Outcomes in Temple syndrome may be improved by aggregation of knowledge, development of targeted, multidisciplinary, life-long care, and education of health professionals to enable patients with Temple syndrome to access earlier diagnosis and therefore better clinical management.

Ethics

Informed Consent: Informed consent for this publication was obtained from the patient and the family.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Sara Stoyanova, Mari Hachmeriyan, Concept: Violeta Iotova, Design: Violeta Iotova, Data Collection or Processing: Violeta Iotova, Sara Stoyanova, Analysis or Interpretation: Nikolinka Yordanova, Literature Search: Nikolinka Yordanova, Violeta Iotova, Writing: Nikolinka Yordanova, Violeta Iotova, Deborah J. G. Mackay, I. Karen Temple.

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References

1. Temple IK, Cockwell A, Hassold T, Pettay D, Jacobs P. Maternal uniparental disomy for chromosome 14. J Med Genet. 1991;28:511-514.
2. Gillessen-Kaesbach G, Albrecht B, Eggermann T, Elbracht M, Mitter D, Morlot S, van Ravenswaaij-Arts CMA, Schulz S, Strobl-Wildemann G, Buiting K, Beygo J. Molecular and clinical studies in 8 patients with Temple syndrome. Clin Genet. 2018;93:1179-1188. Epub 2018 Mar 25
3. Beygo J, Mertel C, Kaya S, Gillessen-Kaesbach G, Eggermann T, Horsthemke B, Buiting K. The origin of imprinting defects in Temple syndrome and comparison with other imprinting disorders. Epigenetics. 2018;13:822-828.
4. Temple IK, Shrubb V, Lever M, Bullman H, Mackay DJ. Isolated imprinting mutation of the DLK1/GTL2 locus associated with a clinical presentation of maternal uniparental disomy of chromosome 14. J Med Genet. 2007;44:637-640. Epub 2007 Jun 29
5. Buiting K, Kanber D, Martín-Subero JI, Lieb W, Terhal P, Albrecht B, Purmann S, Gross S, Lich C, Siebert R, Horsthemke B, Gillessen-Kaesbach G. Clinical features of maternal uniparental disomy 14 in patients with an epimutation and a deletion of the imprinted DLK1/GTL2 gene cluster. Hum Mutat. 2008;29:1141-1146.
6. Briggs TA, Lokulo-Sodipe K, Chandler KE, Mackay DJ, Temple IK. Temple syndrome as a result of isolated hypomethylation of the 14q32 imprinted DLK1/MEG3 region. Am J Med Genet A. 2016;170:70-175. Epub 2015 Sep 23
7. Kagami M, Yanagisawa A, Ota M, Matsuoka K, Nakamura A, Matsubara K, Nakabayashi K, Takada S, Fukami M, Ogata T. Temple syndrome in a patient with variably methylated CpGs at the primary MEG3/DLK1:IG-DMR and severely hypomethylated CpGs at the secondary MEG3:TSS-DMR. Clin Epigenetics. 2019;11:42.
8. Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014;51:495-501. Epub 2014 Jun 2
9. Stalman SE, Kamp GA, Hendriks YM, Hennekam RC, Rotteveel J. Positive effect of growth hormone treatment in maternal uniparental disomy chromosome 14. Clin Endocrinol (Oxf). 2015;83:671-676. Epub 2015 Jul 28
10. Kagami M, Nagasaki K, Kosaki R, Horikawa R, Naiki Y, Saitoh S, Tajima T, Yorifuji T, Numakura C, Mizuno S, Nakamura A, Matsubara K, Fukami M, Ogata T. Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. Genet Med. 2017;19:1356-1366. Epub 2017 May 31
11. Luk HM. Temple syndrome misdiagnosed as Silver-Russell syndrome. Clin Dysmorphol. 201;25:82-83.
12. Hoffmann K, Heller R. Uniparental disomies 7 and 14. Best Pract Res Clin Endocrinol Metab. 2011;25:77-100.
13. Kimura T, Kagami M, Matsubara K, Yatsuga S, Mukasa R, Yatsuga C, Matsumoto T, Koga Y. Temple syndrome diagnosed in an adult patient with clinical autism spectrum disorder. Clin Case Rep. 2018;7:15-18.
14. Mackay D, Bliek J, Kagami M, Tenorio-Castano J, Pereda A, Brioude F, Netchine I, Papingi D, de Franco E, Lever M, Sillibourne J, Lombardi P, Gaston V, Tauber M, Diene G, Bieth E, Fernandez L, Nevado J, Tümer Z, Riccio A, Maher ER, Beygo J, Tannorella P, Russo S, de Nanclares GP, Temple IK, Ogata T, Lapunzina P, Eggermann T. First step towards a consensus strategy for multi-locus diagnostic testing of imprinting disorders. Clin Epigenetics. 2022;14:143.
15. Mitter D, Buiting K, von Eggeling F, Kuechler A, Liehr T, Mau-Holzmann UA, Prott EC, Wieczorek D, Gillessen-Kaesbach G. Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Detection of 10 new patients by methylation-specific PCR. Am J Med Genet A. 2006;140:2039-2049.
16. Lande A, Kroken M, Rabben K, Retterstøl L. Temple syndrome as a differential diagnosis to Prader-Willi syndrome: Identifying three new patients. Am J Med Genet A. 2018;176:175-180. Epub 2017 Nov 21
17. Azzi S, Salem J, Thibaud N, Chantot-Bastaraud S, Lieber E, Netchine I, Harbison MD. A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. J Med Genet. 2015;52:446-453. Epub 2015 May 7
18. Goto M, Kagami M, Nishimura G, Yamagata T. A patient with Temple syndrome satisfying the clinical diagnostic criteria of Silver-Russell syndrome. Am J Med Genet A. 2016;170:2483-2485. Epub 2016 Jun 30
19. Hosoki K, Kagami M, Tanaka T, Kubota M, Kurosawa K, Kato M, Uetake K, Tohyama J, Ogata T, Saitoh S. Maternal uniparental disomy 14 syndrome demonstrates prader-willi syndrome-like phenotype. J Pediatr. 2009;155:900-903. Epub 2009 Oct 1
20. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. J Clin Endocrinol Metab. 2007;92:804-810. Epub 2007 Jan 2
21. Brightman DS, Lokulo-Sodipe O, Searle BA, Mackay DJG, Davies JH, Temple IK, Dauber A. Growth Hormone Improves Short-Term Growth in Patients with Temple Syndrome. Horm Res Paediatr. 2018;90:407-413. Epub 2019 Mar 5
22. Nordenström A, Falhammar H. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency. Eur J Endocrinol. 2019;180:127-145.
23. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, Canton AP, Chrzanowska KH, Davies JH, Dias RP, Dubern B, Elbracht M, Giabicani E, Grimberg A, Grønskov K, Hokken-Koelega AC, Jorge AA, Kagami M, Linglart A, Maghnie M, Mohnike K, Monk D, Moore GE, Murray PG, Ogata T, Petit IO, Russo S, Said E, Toumba M, Tümer Z, Binder G, Eggermann T, Harbison MD, Temple IK, Mackay DJ, Netchine I. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 2017;13:105-124. Epub 2016 Sep 2
24. Goldstone AP, Holland AJ, Hauffa BP, Hokken-Koelega AC, Tauber M; speakers contributors at the Second Expert Meeting of the Comprehensive Care of Patients with PWS. Recommendations for the diagnosis and management of Prader-Willi syndrome. J Clin Endocrinol Metab. 2008;93:4183-4197. Epub 2008 Aug 12 Erratum in: J Clin Endocrinol Metab. 2010;95:5465.

CASE REPORT

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Long-term Growth Hormone Therapy in a Patient with *IGF1R* Deletion Accompanied by Delayed Puberty and Central Hypothyroidism

Nur Berna Çelik[1], Monique Losekoot[2], Emregül Işık[1], E. Nazlı Gönç[1], Ayfer Alikaşifoğlu[1], Nurgün Kandemir[1], Z. Alev Özön[1]

[1]Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Ankara, Turkey
[2]Leiden University Medical Centre, Department of Clinical Genetics, Leiden, The Netherlands

What is already known on this topic?

Insulin-like growth factor-1 (IGF-1) is the main driver of growth during prenatal life. Patients with IGF-1 receptor (IGF1R) defects exhibit variable phenotypic features. The most common symptom is pre- and post-natal growth retardation, followed by microcephaly, developmental delay, facial dysmorphism and extremity anomalies. Recombinant human growth hormone (rhGH) has been used in patients with IGF1R defects with variable treatment response.

What this study adds?

Long-term rhGH with an early initiation may have more beneficial effects in terms of induction of growth. Regarding the complex physiological effects of IGF1, patients should be followed for hormone deficiencies, such as hypogonadism and hypothyroidism.

Abstract

Insulin-like growth factor-1 (IGF-1) is the main driver of growth during prenatal life and acts through IGF-1 receptor (IGF1R). Patients with IGF1R defects exhibit variable phenotypic features. A 10.9-year-old boy presented with severe short stature, microcephaly, minor dysmorphic features and mental retardation. Genetic analysis for *IGF1R* revealed heterozygous deletion of the complete *IGF1R*. At the age of 12.3 years, daily subcutaneous recombinant human growth hormone (rhGH) was started and continued for a total of 5.7 years in two courses with improvement of height velocity as well as final height. Puberty was delayed and eventually he did not achieve full puberty, suggesting partial hypogonadotropic hypogonadism. Hypothyroidism initially developed during rhGH therapy. However, low T4 levels persisted after cessation of rhGH therapy and thus central hypothyroidism is a likely diagnosis. rhGH has partial effect for induction of growth in cases with *IGF1R* defects. However, long-term treatment with an early initiation may have more beneficial effects. In addition, patients with *IGF1R* defects should be followed for delayed puberty-hypogonadism, and hypothyroidism.
Keywords: IGF1R, deletion, growth hormone therapy, delayed puberty, hypothyroidism

Introduction

Growth factors are crucial for prenatal growth. Insulin-like growth factor-1 (IGF-1), which has structural homology with proinsulin, is the main driver of growth during prenatal life and acts through IGF-1 receptor (IGF1R). The gene coding for IGF1R, *IGF1R*, is located on the distal part on the long arm of the chromosome 15 (15q26.3) (1). In animal models, *IGF1R* null mice exhibited severe growth restriction (45% of normal size) and died soon after birth due to lung and

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Address for Correspondence: Nur Berna Çelik MD, Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Ankara, Turkey
E-mail: n.b.celik@hotmail.com **ORCID:** orcid.org/0000-0001-5945-286X

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respiratory muscle hypoplasia, ossification was delayed, epidermis was underdeveloped and there were central nervous system anomalies (2). Since the first description of patients with *IGF1R* mutation who had intrauterine growth retardation, poor postnatal growth, developmental delay and microcephaly, cumulative evidence has shown that the phenotypic characteristics of patients with *IGF1R* defects can vary widely (3,4,5). 15q26 terminal deletions lead to contiguous gene syndrome and there is no clear genotype-phenotype correlation with significant inter- and intra-familial variability. Homozygous or compound heterozygous mutations seem to cause more severe phenotype (3,6,7). Endocrine consequences of *IGF1R* defects other than short stature, such as delayed puberty, premature ovarian failure, and growth hormone deficiency have been reported very rarely (5,8,9,10,11). Central adrenal insufficiency and hypothyroidism have not been reported previously.

Recombinant human growth hormone (rhGH) is approved by the Food and Drug Administration and the European Medicine Agency for treatment of children born small for gestational age (SGA). rhGH has been used in patients with *IGF1R* defects with variable treatment response. rhGH may be discontinued due to no improvement in growth velocity, continued without catch-up growth (3,10,12,13,14,15) or result in mild catch-up growth (13,14,16).

We report a boy with 15q terminal deletion, who presented with severe growth retardation, microcephaly, and developmental delay who also had delayed puberty and central hypothyroidism. We also aim to report the long-term results of rhGH therapy.

Case Report

A 10.9-year-old boy was referred for short stature. He was born at term to healthy, nonconsanguineous parents with a reportedly normal birth weight but his birth length was unknown. Maternal and paternal heights were -2.8 standard deviation score (SDS) (146.7 cm) and -2.0 SDS (163.7 cm), respectively, and midparental height was -2.3 SDS (161.7 cm). There was no feeding difficulty during infancy. He was able to say his first words and walk at the age of 1.5 and 2.5 years, respectively. He had been evaluated at another health center for short stature at the age of six years and thyroid hormones, growth factors, and celiac antibodies were normal.

He presented with a height of -5.3 SDS (108.3 cm), a weight of -4.9 SDS (18.5 kg), and a head circumference of -4.1 SDS (48 cm) at the age of 10.9 years old. He had proportional severe short stature with no dysmorphic features, except for a triangular face. He was prepubertal. Neurological examination was unremarkable, except for mental retardation detected in the Wechsler Intelligence Scale for Children-Revised test (intelligence quotient 65). Bone age determined by the Greulich and Pyle method was five years. Total blood count and blood chemistry were normal, as was a skeletal survey. In the endocrine work-up, IGF-1 was 240.2 ng/mL (close to 0 SDS), insulin-like growth factor binding protein-3 was 4228.7 ng/mL (0.43 SDS). GH stimulation test showed a peak GH response of 10.3 ng/mL. Other pituitary hormones, including adrenocorticotropic hormone (ACTH) [19.5 pg/mL (normal range 0-46)], cortisol (8.5 µg/dL), prolactin [9.3 ng/mL (normal range 2.5-18)], free T4 [1.19 ng/dL (normal range 0.9-1.7)], and thyroid stimulating hormone (TSH) [2.4 uIU/mL (normal range 0.3-4.2)] were normal. He had primary nocturnal enuresis and renal ultrasound revealed kidney size in the lower range for age.

Karyotype analysis was 46,XY. Genetic analysis for *IGF1R* was performed in Leiden University Medical Centre. MLPA analysis of the coding region (exon 1-21) revealed heterozygous deletion of the complete *IGF1R* gene. SNP microarray identified a 3.3 Mb terminal deletion on the long arm of the chromosome 15 (15q26.3x1) which included *IGF1R* and there was also a terminal duplication with a maximum size of 2.6 Mb on the short arm of chromosome 9 (9p24.3p24.2x3). The terminal 9p duplication and the terminal 15q deletion suggest the presence of an unbalanced reciprocal translocation between the short arm of chromosome 9 and the long arm of chromosome 15. MLPA and SNP microarray were normal for chromosome 9 and 15 in both mother and father.

At the age of 12.3 years, height was -5.27 SDS (114.2 cm), weight -5.4 SDS (19.5 kg) and growth velocity 4.1 cm/year. His IGF-1 was 383 mg/dL (0.5 SDS). Daily subcutaneous rhGH was started at a dose of 0.21 mg/kg/week (Figure 1) and the dose was increased to 0.30 mg/kg/week after three months. Treatment was continued for eighteen months. During this period his growth velocity was 7.1 cm for the first year (height was 121.3 cm, -4.67 SDS), which then slowed to 2.2 cm during the next six months. Treatment was withdrawn for slow growth velocity. Nine months later, rhGH treatment was restarted at a dose of 0.30 mg/kg/week due to slow growth rate (2.9 cm in nine months). During rhGH therapy, serum IGF-1 levels varied between + 1 and + 2 SDS.

At the age of 14.8 years, testes were 6 mL bilaterally and increased to 8 mL at the age of 15.7 years, but did not progress afterwards. At the age of 16.5 years with an observation of delayed puberty [testes volumes 8 mL bilaterally, follicle-stimulating hormone (FSH) 5.27 mIU/mL (normal range 1.3-19.2), luteinizing hormone (LH) 0.21 mIU/mL (normal range 1.8-8.6), testosterone 29.4 ng/dL (normal range 220-800)] intramuscular testosterone (propionate 30

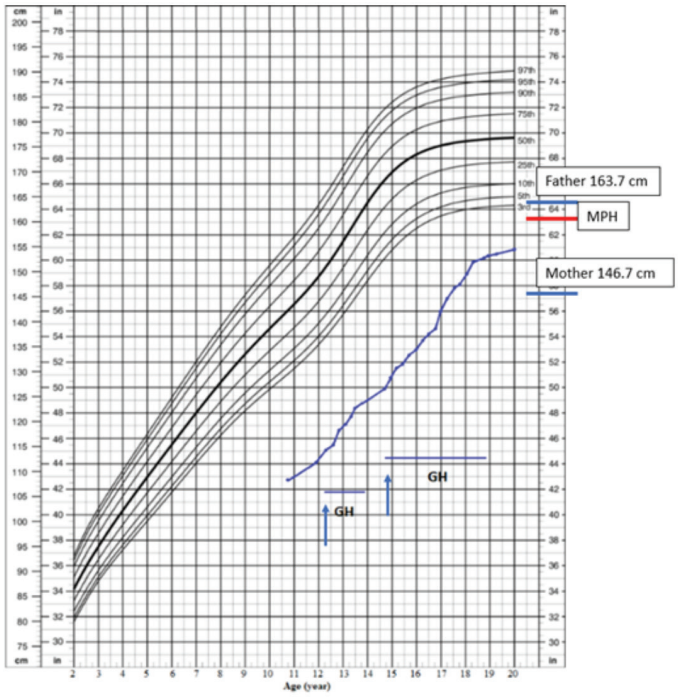

Figure 1. Growth chart of the patient. The patient had two courses of growth hormone therapy. Normative data for boys is from the Centers for Disease Control and Prevention

GH: growth hormone

mg, phenylpropionate 60 mg, isocaproat 60 mg, decanoate 100 mg) was commenced at a dose of 50 mg/monthly.

At the age of 17.3 years, while he was on rhGH, thyroid function tests revealed hypothyroidism [TSH 2.13 uIU/mL, (normal range 0.4-5.3) with free T4 0.51 ng/dL, (normal range 0.6-1.1)]. Central adrenal insufficiency was also diagnosed [ACTH 32.5 pg/mL, peak cortisol during low dose ACTH stimulation test was 16.8 µg/dL (N: > 18.9)]. Thus, both hydrocortisone and levothyroxine were started. On pituitary magnetic resonance imaging, the height of the pituitary gland was 4.5 mm, and a pars intermedia cyst 2 mm in diameter was present on the anterior region of neurohypophysis.

At the age of 18.8 years, his height was -3.26 SDS (152.9 cm) and weight was -3.44 SDS (44.6 kg). His height increased 0.8 cm in the last 6 months, bone age was 16 years and rhGH treatment was withdrawn. After 5.7 years of rhGH treatment in two courses, he had a height gain of 2.01 SDS. Testis volumes increased to 10 mL bilaterally [FSH 7.1 mIU/mL (normal range 0.9-11.9), LH 1.5 mIU/mL (normal range 0.5-12.0), testosterone 152.2 ng/dL (normal range 151-794) two weeks after the last dose of testosterone], and testosterone treatment was withdrawn as well. In addition, the hypothalamic-pituitary-adrenal axis was rechecked, the peak cortisol response to low dose ACTH test was 18.9 µg/dL and hydrocortisone was discontinued with instructions for stress coverage. At the age of 19.5 years the size of his testes and testosterone concentrations had not increased [testes 10 mL bilaterally, FSH 8.05 mIU/mL (normal range 0.9-11.9), LH 0.84 mIU/mL (normal range 0.6-12.1), testosterone 156.9 ng/dL (normal range 47-981)], so testosterone was restarted. During the last follow-up when he was 20 years old, height was -3.06 SDS (154.8 cm), weight 45 kg (-3.45 SDS), and he had not been using levothyroxine for three months. Thyroid function test still suggested central hypothyroidism [TSH 3.8 mIU/mL, (normal range 0.27-4.2); free T4 0.88 ng/dL, (normal range 0.93-1.7), and free T3 3.26 ng/L (2-4.4)].

Discussion

We report a boy with *IGF1R* deletion presenting with severe short stature, microcephaly, mental retardation and mild dysmorphic features. Growth hormone therapy for a total of 5.7 years in two courses improved height velocity, as well as final height. Moreover, puberty was arrested and eventually he could not complete puberty, suggesting partial hypogonadotropic hypogonadism. Hypothyroidism developed during GH therapy which may be associated with isolated GH deficiency during GH therapy (17). However, low T4 levels persisted after cessation of GH therapy and thus central hypothyroidism is a likely diagnosis.

Patients with *IGF1R* defects exhibit variable phenotypic features. The most common symptom is pre- and post-natal growth retardation, followed by microcephaly, developmental delay, facial dysmorphism and anomalies of the extremities. Although birth weight or height below -2 SDS were used as the inclusion criteria in studies evaluating *IGF1R* defects (15), patients have shown wide variation in these parameters; birth weight between -4.1 and -0.8, birth length -5.8 and -1.0, and head circumference -5.7 and 0.8 SDS (3,6,12,14). Patients with terminal 15q deletions may exhibit additional features involving other organ systems, such as cardiac, genitourinary, respiratory, and ocular (7,14) disorders, attributed to defects in contiguous genes, some of which may impact growth. However, the presented patient had only mild dysmorphic features, neurodevelopmental delay, and small-normal kidneys without involvement of other major organ systems.

rhGH therapy has been recommended for patients with *IGFR1* defects in higher than usual doses to overcome receptor resistance (15). Growth promoting effects of rhGH is less pronounced in comparison to patients with SGA, and response is quite variable among patients with *IGF1R* defects (18). Although the rhGH response in the first year is lower than in SGA patients, the constant growth velocity in the following years may suggest the importance of

long-term treatment (18). The dose of rhGH is expected to be important, however, Göpel et al. (18) did not find any association between dose and treatment response. In addition, it has been a matter of debate whether genotype influences rhGH treatment response. Walenkamp et al. (4) found no difference in 3-year rhGH response between twelve patients with mutations and seven with deletions who received rhGH therapy at similar ages. Göpel et al. (18) reported that the ratio of patients with a good response to treatment was higher in carriers of mutations within the intracellular part of IGF1R compared to the extracellular part. However, it should be emphasized that number of patients were limited due to the rarity of *IGF1R* defects.

We reviewed 28 patients with *IGF1R* defects who received rhGH (Table 1). Thirteen (46%) had terminal 15q deletions or ring chromosome, fourteen (50%) heterozygous mutations, and one (4%) compound heterozygous mutation. Sixty-one percent of patients with terminal 15q deletions or ring chromosome, and 35% of patients with *IGF1R* mutations exhibited ≥0.5 SDS increase in height during the first year of rhGH. Sixty-nine percent of patients with terminal 15q deletions or ring chromosome, and 42% of patients with mutations had a height gain of ≥1 SDS based on the last evaluation or final height. One of the two patients with the worst treatment response had a compound heterozygous mutation and the other with 15q26 deletion had hypoplastic left heart. Forty-six percent of patients who did not gain ≥0.5 SDS in the first year of treatment achieved ≥1 SDS with prolonged treatment. The presented patient had a height gain of 0.6 SDS in the first year of rhGH treatment, and 2.01 SDS overall with a 5.7 year treatment duration.

The presence of IGF-1 and IGF-1R has been demonstrated in various cells, including the pituitary (19). IGF-1 is a mitogenic hormone that induces proliferation and differentiation of various cells and participates in physiological regulation. IGF-1 is the key modulator of GH action and also participates in regulation of the hypothalamo-pituitary-gonad (HPG) axis. The expression of GH and IGF1 receptors in the elements of HPG axis and reproductive organs has been demonstrated in molecular studies. GH and IGF1 participate in various stages of maturation of the reproductive axis, including intrauterine stages, mini-puberty and onset of puberty. Cryptorchidism was reported in two patients with *IGF1R* deletion (7,20). Although contiguous gene syndrome cannot be excluded as an etiology of cryptorchidism, IGF1R haploinsufficiency could still be the causative factor, highlighting intrauterine effects. *In vitro* animal models showed that IGF-1 both induces proliferation of gonadotrophs and secretion of gonadotropins (21), which underlines the importance of growth factors for induction of puberty and its progression. IGF-1 participates in testis and ovary function, in terms of Sertoli and granulosa cell survival and production of gonadal steroid hormones (22,23). Hypergonadotropic hypogonadism was also reported in patients with *IGF1R* defects (5,24). It is interesting that cases with *IGF1R* duplication had azoospermia. These data suggest that an intact IGF-1 system is necessary for the maturation and maintenance of the reproductive system. The presented patient exhibited features of hypogonadotropic hypogonadism. Puberty started at the age of 14.8 years, and did not progress appropriately, so sex steroid replacement was established. Patients with delayed puberty were reported previously with *IGF1R* defects, but none of them required sex steroids since puberty progressed spontaneously (8,9).

One of the consequences of rhGH therapy is alteration of thyroid hormones. GH induces the activity of deiodinase, probably type 2, thus free T4 level may decrease and free T3 level may increase during rhGH therapy. TSH concentration is not expected to increase in the face of increased free T3. Thus rhGH therapy can either mimic or unmask central hypothyroidism (17). Our patient developed central hypothyroidism during rhGH therapy, however, this condition continued even after cessation of rhGH. Since *IGF1R* expressed in pituitary somatotrophs participates in negative feedback on the somatotropic axis, receptor resistance may disrupt negative feed-back leading to an increase in GH levels. Elevation in GH levels may induce somatostatin from the hypothalamus which is a weak inhibitor of TSH (25). Studies using salmon pituitary cells have shown that IGF-1 can stimulate thyrotropin beta subunit transcript in a dose-dependent manner (26). In addition, the GH-IGF1 axis has important effects on thyrocytes. In *in vitro* animal studies, GH and IGF-1 showed synergistic effects with TSH on thyroid gland growth and hormone production (27). Thus, *IGF1R* defects may be expected to impact thyroid function at multiple levels. Interestingly, no patient with an *IGF1R* defect and hypothyroidism has been reported to date. Thus, it is not possible to ensure that alterations in thyroid function in the current patient is a direct consequence of IGF-1 resistance. Also, a pars intermedia cyst was detected on MRI. Pars intermedia cysts, remnants of Rathke's pouch, rarely causes symptoms, with symptoms being related to mass effect and/or pituitary hormone deficiency (28). Some reports suggested a positive correlation between cyst size and impairment of pituitary function (29), while other reports suggest an association between symptoms and chronic inflammation around the cyst wall (28). However, small cysts are expected to be asymptomatic and detected incidentally or at autopsy (29), and the frequency of pituitary hormone deficiency increases in ≥10 mm cysts (30). Therefore, pituitary hormone deficiency would not be expected in a 2 mm diameter pars intermedia cyst.

Table 1. Features of genotype, auxology and phenotype of patients who were given rhGH

Author (year)	Deletion/mutation	Gender (F/M)	Birth weight/ length/head circumference	Age at first evaluation; height (SDS)	Age at the start of rhGH (year); height (SDS)	Duration (year)	Height gain at the first year of rhGH (SDS)	Age (year) at last evaluation; height (SDS)	Final height	Other features	Affected family member	Other hormonal deficiencies
Ho et al. (31)	46,XX,r(15) (p11q26.3)	F	-0.8 / NA / NA	0.4; -5.6	1.8; -5.3	12.4	+1	NA; -2.1	-2.1	DD	NA	NA
Ho et al. (31)	46,XX,r(15) (p13q26.2)	F	-1.6 / NA / -3.3	0.5; -4.4	2.6; -3.3	12.2	+0.5	NA; -2.5	-2.5	Hip dislocation, DD	NA	NA
Ho et al. (31)	46,XY,del(15) (q26.3)	M	-1.6 / NA / NA	1.5; -3.7	5.2; -4.0	11.8	+0.7	NA; -2.6	-2.6	Bilateral talipes, DD	NA	NA
Ester et al. (10)	15q26.3 deletion, exons 3-21	M	-1.9 / -2.2 / NA	3.0; -3.84	7; -3.57	10	+0.83	17; -1.89	NA	DD, DF, EA, HL	No	NA
Gkourogianni et al. (32)*	c.3364G > T p.Gly1122Cys	M	NA	NA	9.1; -3.5	9.6	+0.2	18.7; -1.8	-1.8 SDS	No DF.	Mother; -2.1 SDS	DP
Abuzzahab et al. (3)	Compound heterozygousArg108Gln, Lys115Asn, exon 2	F	-3.5 / NA / NA	NA	4.1 yr, for 2 years and at 8.7 yr old restarted	7.9 yrs in two courses	+0.17 in the first course	NA	-4.8 SDS	DD and psychiatric disorders		Menarche at the age of 12.5 yr
Walenkamp et al. (13)	15q26.2-qter	F	-3 / -1.3 / -2	4.5; -3.5	5.3; NA	6.7	NA	NA	-1.6 SDS	DD	No	NTF. Puberty started at the age of 12.8 yr
Yang et al. (33)*	c.3740T>C, p.M1247T	F	-1.9 / NA / NA	2.1; -3.85	2.8; -3.36	6	+0.6	8.8; -2.4	NA	No DD	Mother; -1.96 SDS	NA
Ho et al. (31)	46,XX,del(15) (q26.2)	F	-2.9 / NA / -3.7	3.0; -4.9	5.0; -4.9	5.8	+0.1	NA; -4.9	-4.9	Hypoplastic left heart, DD, dysplastic kidney	NA	NA
Leal et al. (15)	c.1531C>T, p.Arg511Trp, exon 7	F	-2.5 / NA / NA	5.8; -2.7	8.4; -2.9	5	+0.9	13.3; -1.7	NA	No DD Mild DF	Mother; -2.9 SDS	NP
Veenma et al. (14)**	15q26.3 microdeletion	M	-1.7 / -2.3 / 0.88	12; -4.2	13.8; NA	4.6	+0.1	NA, the overall catch-up growth was +1.8 SDS	NA	DD, DF, café-au-lait, strabismus, refraction anomalies of lens	Mother; -4.42 SDS	NP
Labarta et al. (12)	c.1549A>T, p.Y487F, exon 7	F	-3.46 / -4.9 / -5.7	1.5; -2.84	3.4; -3.19	4.1	+0.31	7.5; -2.39	NA	Slightly retarded	Mother; -1.6 SDS	Mother menarche at the age of 13 yr
Ester et al. (10)	15q26.3 deletion, exons 1-21	F	-1.28 / -2.21 / NA	2.3; -3.46	4; -3.42	4	+1.02	8.3; -1.68	NA	DD, DF, EA, HL, hyperlaxity	No	NA

Table 1. Continued

Author (year)	Deletion/mutation	Gender (F/M)	Birth weight/ length/head circumference	Age at first evaluation; height (SDS)	Age at the start of rhGH (year); height (SDS)	Duration (year)	Height gain at the first year of rhGH (SDS)	Age (year) at last evaluation; height (SDS)	Final height	Other features	Affected family member	Other hormonal deficiencies
Nuutinen et al. (34)	46,XY, r(15) (pll.2q26.2)		-3.2 / -4.0 / NA	0.6; -6.2	2.2; -6.2	2	+1.2	4.2; -4.4	NA	DD, DF, café-au-lait	NA	NTF
Choi et al. (7)*	c.420del, p.Ala110fsX20, in exon 2	F	-2.1 / NA / NA	6.8; -3.56	8; -3.56	1	+1.18	9; -2.38	NA	No DD or DF	Father; -4.19 SDS	NTF
Ho et al. (31)	46,XX,r(15) (p13q26.3)	F	-2.5 / NA / -2.0	0.5; -3.0	3.3; -3.8	1.0	+0.8	4.3; -3.0	NA	DD, DF	NA	NA
Choi et al. (7)*	c.420del, p.Ala110fsX20, in exon 2	M	-1.96 / NA / NA	9.5; -3.47	10.5; -3.42	1	+0.64	11.5; -2.78	NA	NA	Father; -4.19 SDS	NA
Ho et al. (31)	46,XY,del(15) (q26.3)	M	-1.4 / NA / NA	3.2; -4.8	6.2; -4.6	1.6	+0.6	7.8; -3.4	NA	DD, DF	NA	NA
Raile et al. (35)	Arg59Ter, exon 2	M	-3.5 / -5.8 / NA	1.1; -3.8	6.4; -2.51	2	+0.55	8.5; -1.5	NA	DD, DF	Mother; -2.6 SDS	NTF, NAF, normal prolactin
Wallborn et al. (36)*	c.1886T > A, p. V599E	F	-2.26 / -1.82 / < 3p	NA	7.42; -2.27	1.5	+0.43	9.02; -1.4	NA	DD, ADHD	Mother; -3.3 SDS	NTF
Mahmoud et al. (16)*	15q26.2q26.3 deletion	M	-3 / -3.2 / NA	2.5; -9.3	3.4; -3.4	2.6	+0.33	6; -1.5	NA	Mild DD, DF	No	NTF
Gkourogianni et al. (32)*	c.3364G > T p.Gly1122Cys	M	-2.0 / -1.6 / -2.15	6.8; -2.3	7.9; -2.2	2.6	+0.23	10.5; -1.15	NA	Attention deficit disorder	Father; -1.8 SDS	NA
Ho et al. (31)	46,XX,del(15) t(15;16) (q26.1:q22.3)	F	-1.6 / NA / NA	5.0; -5.4	12.4; -5.9	2.2	+0.2	14.6; -5.7	NA	DD, EA, VSD, subglottic stenosis	NA	NA
Fang et al. (37)*	19Dup in exon 18	M	-3.04 / -1.5 / NA	9.6; -3.6	10; -3.65	2	+0.03	12; -3.05	NA	Bifid uvula, ADHD	Mother; -4.6 SDS	NTF, NAF
Inagaki et al. (38)	c.1577G > A, p.R481Q, exon 7	F	-3.1 / -4.9 / NA	13.6; -5	NA	0.5	0 SDS	NA	NA	Mild DF	Mother; -5.7 SDS	T2P2 at presentation
Mohn et al. (39)	c.1161C > A, p.Tyr387X, exon 5	M	-2.03 / -3.08 / NA	4; -4.58	8; NA	1	No improvement in GV	18; -3.08	NA	No DD	Father; -2.94 SDS	NP
Kawashima et al. (40)	c.3405C > A	F	-1.5 / -2.5 / NA	6; -3.0	6; -3.0	3	NA	9; -1.5	NA	DD	Mother; -4.0 SDS	NA
Kawashima et al. (40)	c.1382G > T, R431L	F	-1.8 / -3.2 / NA	3; -2.9	5; -3.0	2	NA	8; -2.7	NA	No DD	Mother; -1.2 SDS	NA
Fujimoto et al. (41)	c.3798C > T, p.Q1250X, exon 21	M	-3.3 / -2.1 / -3.7	3; -3.2	6; -3.1	2	NA	8.7; -2.6 (at the end of the rhGh -2.5)	NA	No DD	No	NA

*GV, height SDS calculated from growth charts. **In the first 2 years also received GnRHa.

ADHD: attention deficit hyperactivity disorder, CA: cardiac anomaly, DD: developmental delay, DF: dysmorphic features, DP: delayed puberty, EA: extremity anomalies, GV: growth velocity, HL: hearing loss, MR: mental retardation, NAF: normal adrenal function, NP: normal puberty, NTF: normal thyroid function, F/M: female/male, rhGH: recombinant human growth hormone, SDS: standard deviation score, yr: year, NA: not applicable

IGF-1 immunoreactivity was detected in the same secretory granules of the corticotroph cells, indicating a concomitant secretion and release of both hormones (19). Despite the coexistence of both hormones, recent studies showed no effect of IGF-1 on ACTH secretion and corticotroph responsiveness to CRH (19). Instead, corticotroph cells may require IGF-1 to protect them against apoptosis, especially in stressful situations (19). The first low dose ACTH test that was performed, before levothyroxine treatment, revealed an inadequate serum cortisol peak, and the results of the second were just above the lowest reference range. We could not definitively exclude the diagnosis of central adrenal insufficiency, due to the technical limitations of the low dose ACTH test and its lower sensitivity and specificity compared to the insulin tolerance test. However, the lack of protective effects of IGF1 could make these patients vulnerable to apoptosis of corticotroph cells.

Conclusion

In conclusion, rhGH has partial beneficial effect on growth in cases with *IGF1R* defects if long-term, early-onset treatment has been instituted. Even if the treatment response to rhGH is relatively poor during the first year, it is important to continue the treatment since 42 % of the patients have a height gain of more than 1 SDS in the long-term. In addition, patients with *IGF1R* defects should be followed for later development of hormone deficiencies.

Ethics

Informed Consent: Written informed consent was collected from the patient.

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Footnotes

Authorship Contributions

Medical Practices: Nur Berna Çelik, Monique Losekoot, Emregül Işık, E. Nazlı Gönç, Ayfer Alikaşifoğlu, Nurgün Kandemir, Z. Alev Özön, Concept: Nur Berna Çelik, E. Nazlı Gönç, Z. Alev Özön, Design: Nur Berna Çelik, E. Nazlı Gönç, Z. Alev Özön, Analysis or Interpretation: E. Nazlı Gönç, Ayfer Alikaşifoğlu, Nurgün Kandemir, Z. Alev Özön, Literature Search: Nur Berna Çelik, E. Nazlı Gönç, Z. Alev Özön, Writing: Nur Berna Çelik, E. Nazlı Gönç, Z. Alev Özön.

Conflict of Interest: One author of this article, Z. Alev Özön is a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, she did not take part in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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References

1. Walenkamp MJ, Losekoot M, Wit JM. Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. Endocr Dev. 2013;24:128-137. Epub 2013 Feb 1
2. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell. 1993;75:59-72.
3. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfäffle R, Raile K, Seidel B, Smith RJ, Chernausek SD; Intrauterine Growth Retardation (IUGR) Study Group. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med. 2003;349:2211-2222.
4. Walenkamp MJE, Robers JML, Wit JM, Zandwijken GRJ, van Duyvenvoorde HA, Oostdijk W, Hokken-Koelega ACS, Kant SG, Losekoot M. Phenotypic Features and Response to GH Treatment of Patients With a Molecular Defect of the IGF-1 Receptor. J Clin Endocrinol Metab. 2019;104:3157-3171.
5. Gonc EN, Ozon ZA, Oguz S, Kabacam S, Taskiran EZ, Kiper POS, Utine GE, Alikasifoglu A, Kandemir N, Boduroglu OK, Alikasifoglu M. Genetic IGF1R defects: new cases expand the spectrum of clinical features. J Endocrinol Invest. 2020;43:1739-1748. Epub 2020 Apr 30
6. Prontera P, Micale L, Verrotti A, Napolioni V, Stangoni G, Merla G. A New Homozygous IGF1R Variant Defines a Clinically Recognizable Incomplete Dominant form of SHORT Syndrome. Hum Mutat. 2015;36:1043-1047. Epub 2015 Aug 24
7. Choi JH, Kang M, Kim GH, Hong M, Jin HY, Lee BH, Park JY, Lee SM, Seo EJ, Yoo HW. Clinical and functional characteristics of a novel heterozygous mutation of the IGF1R gene and IGF1R haploinsufficiency due to terminal 15q26.2->qter deletion in patients with intrauterine growth retardation and postnatal catch-up growth failure. J Clin Endocrinol Metab. 2011;96:130-134. Epub 2010 Oct 20
8. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, Breuning MH, Romijn JA, Karperien M, Wit JM. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. J Clin Endocrinol Metab. 2006;91:3062-3070. Epub 2006 Jun 6
9. Kruis T, Klammt J, Galli-Tsinopoulou A, Wallborn T, Schlicke M, Müller E, Kratzsch J, Körner A, Odeh R, Kiess W, Pfäffle R. Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. J Clin Endocrinol Metab. 2010;95:1137-1142. Epub 2010 Jan 26
10. Ester WA, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CA, Govaerts LC, Wit JM, Hokken-Koelega AC, Losekoot M. Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. J Clin Endocrinol Metab. 2009;94:4717-4727. Epub 2009 Oct 28
11. Yoon JS, Hwang IT. Microdeletion in the IGF-1 receptor gene of a patient with short stature and obesity: a case report. J Pediatr Endocrinol Metab. 2021;34:255-259.
12. Labarta JI, Barrio E, Audí L, Fernández-Cancio M, Andaluz P, de Arriba A, Puga B, Calvo MT, Mayayo E, Carrascosa A, Ferrández-Longás A. Familial short stature and intrauterine growth retardation associated

with a novel mutation in the IGF-I receptor (IGF1R) gene. Clin Endocrinol (Oxf). 2013;78:255-262.

13. Walenkamp MJ, de Muinck Keizer-Schrama SM, de Mos M, Kalf ME, van Duyvenvoorde HA, Boot AM, Kant SG, White SJ, Losekoot M, Den Dunnen JT, Karperien M, Wit JM. Successful long-term growth hormone therapy in a girl with haploinsufficiency of the insulin-like growth factor-I receptor due to a terminal 15q26.2-> qter deletion detected by multiplex ligation probe amplification. J Clin Endocrinol Metab. 2008;93:2421-2425. Epub 2008 Mar 18
14. Veenma DC, Eussen HJ, Govaerts LC, de Kort SW, Odink RJ, Wouters CH, Hokken-Koelega AC, de Klein A. Phenotype-genotype correlation in a familial IGF1R microdeletion case. J Med Genet. 2010;47:492-498. Epub 2009 Dec 2
15. Leal AC, Montenegro LR, Saito RF, Ribeiro TC, Coutinho DC, Mendonca BB, Arnhold IJ, Jorge AA. Analysis of the insulin-like growth factor 1 receptor gene in children born small for gestational age: in vitro characterization of a novel mutation (p.Arg511Trp). Clin Endocrinol (Oxf). 2013;78:558-563.
16. Mahmoud R, Naidu A, Risheg H, Kimonis V. Response to Growth Hormone Treatment in a Patient with Insulin-Like Growth Factor 1 Receptor Deletion. J Clin Res Pediatr Endocrinol. 2017;9:380-386. Epub 2017 Jul 17
17. Kucharska AM, Witkowska-Sędek E, Rumińska M, Pyrżak B. Thyroid Hormone Changes Related to Growth Hormone Therapy in Growth Hormone Deficient Patients. J Clin Med. 2021;10:5354.
18. Göpel E, Rockstroh D, Pfäffle H, Schlicke M, Pozza SB, Gannagé-Yared MH, Gucev Z, Mohn A, Harmel EM, Volkmann J, Weihrauch-Blüher S, Gausche R, Bogatsch H, Beger C, Klammt J, Pfäffle R. A Comprehensive Cohort Analysis Comparing Growth and GH Therapy Response in IGF1R Mutation Carriers and SGA Children. J Clin Endocrinol Metab. 2020;105:dgz165.
19. Eppler E, Jevdjovic T, Maake C, Reinecke M. Insulin-like growth factor I (IGF-I) and its receptor (IGF-1R) in the rat anterior pituitary. Eur J Neurosci. 2007;25:191-200.
20. Rudaks LI, Nicholl JK, Bratkovic D, Barnett CP. Short stature due to 15q26 microdeletion involving IGF1R: report of an additional case and review of the literature. Am J Med Genet A. 2011;155:3139-3143. Epub 2011 Nov 7
21. Huang YS, Rousseau K, Le Belle N, Vidal B, Burzawa-Gérard E, Marchelidon J, Dufour S. Insulin-like growth factor-I stimulates gonadotrophin production from eel pituitary cells: a possible metabolic signal for induction of puberty. J Endocrinol. 1998;159:43-52.
22. Dance A, Kastelic J, Thundathil J. A combination of insulin-like growth factor I (IGF-I) and FSH promotes proliferation of prepubertal bovine Sertoli cells isolated and cultured in vitro. Reprod Fertil Dev. 2017;29:1635-1641.
23. Monte APO, Barros VRP, Santos JM, Menezes VG, Cavalcante AYP, Gouveia BB, Bezerra MES, Macedo TJS, Matos MHT. Immunohistochemical localization of insulin-like growth factor-1 (IGF-1) in the sheep ovary and the synergistic effect of IGF-1 and FSH on follicular development in vitro and LH receptor immunostaining. Theriogenology. 2019;129:61-69. Epub 2019 Feb 8
24. O'Riordan AM, McGrath N, Sharif F, Murphy NP, Franklin O, Lynch SA, O'Grady MJ. Expanding the clinical spectrum of chromosome 15q26 terminal deletions associated with IGF-1 resistance. Eur J Pediatr. 2017;176:137-142. Epub 2016 Nov 8
25. Takano K, Ajima M, Teramoto A, Hata K, Yamashita N. Mechanisms of action of somatostatin on human TSH-secreting adenoma cells. Am J Physiol. 1995;268:558-564.
26. Fleming MS, Maugars G, Martin P, Dufour S, Rousseau K. Differential Regulation of the Expression of the Two Thyrotropin Beta Subunit Paralogs by Salmon Pituitary Cells In Vitro. Front Endocrinol (Lausanne). 2020;11:603538.
27. Feldt-Rasmussen U. Interactions between growth hormone and the thyroid gland -- with special reference to biochemical diagnosis. Curr Med Chem. 2007;14:2783-2788.
28. Nishioka H, Haraoka J, Izawa H, Ikeda Y. Magnetic resonance imaging, clinical manifestations, and management of Rathke's cleft cyst. Clin Endocrinol (Oxf). 2006;64:184-188.
29. Trifanescu R, Ansorge O, Wass JA, Grossman AB, Karavitaki N. Rathke's cleft cysts. Clin Endocrinol (Oxf). 2012;76:151-160.
30. Dadej D, Skraba K, Matyjaszek-Matuszek B, Świrska J, Ruchała M, Ziemnicka K. Presenting symptoms and endocrine dysfunction in Rathke cleft cysts - a two-centre experience. Endokrynol Pol. 2021;72:505-511.
31. Ho SC, Clayton P, Vasudevan P, Greening J, Wardhaugh B, Shaw N, Kelnar C, Kirk J, Högler W. Recombinant Human Growth Hormone Therapy in Children with Chromosome 15q26 Deletion. Horm Res Paediatr. 2015;83:424-430.
32. Gkourogianni A, Andrade AC, Jonsson BA, Segerlund E, Werner-Sperker A, Horemuzova E, Dahlgren J, Burstedt M, Nilsson O. Pre- and postnatal growth failure with microcephaly due to two novel heterozygous IGF1R mutations and response to growth hormone treatment. Acta Paediatr. 2020;109:2067-2074. Epub 2020 Mar 6
33. Yang L, Xu DD, Sun CJ, Wu J, Wei HY, Liu Y, Zhang MY, Luo FH. IGF1R Variants in Patients With Growth Impairment: Four Novel Variants and Genotype-Phenotype Correlations. J Clin Endocrinol Metab. 2018;103:3939-3944.
34. Nuutinen M, Kouvalainen K, Knip M. Good growth response to growth hormone treatment in the ring chromosome 15 syndrome. J Med Genet. 1995;32:486-487.
35. Raile K, Klammt J, Schneider A, Keller A, Laue S, Smith R, Pfäffle R, Kratzsch J, Keller E, Kiess W. Clinical and functional characteristics of the human Arg59Ter insulin-like growth factor i receptor (IGF1R) mutation: implications for a gene dosage effect of the human IGF1R. J Clin Endocrinol Metab. 2006;91:2264-2271. Epub 2006 Mar 28
36. Wallborn T, Wüller S, Klammt J, Kruis T, Kratzsch J, Schmidt G, Schlicke M, Müller E, van de Leur HS, Kiess W, Pfäffle R. A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. J Clin Endocrinol Metab. 2010;95:2316-2324. Epub 2010 Mar 31
37. Fang P, Schwartz ID, Johnson BD, Derr MA, Roberts CT Jr, Hwa V, Rosenfeld RG. Familial short stature caused by haploinsufficiency of the insulin-like growth factor i receptor due to nonsense-mediated messenger ribonucleic acid decay. J Clin Endocrinol Metab. 2009;94:1740-1747. Epub 2009 Feb 24
38. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S, LeRoith D. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab. 2007;92:1542-1548. Epub 2007 Jan 30
39. Mohn A, Marcovecchio ML, de Giorgis T, Pfaeffle R, Chiarelli F, Kiess W. An insulin-like growth factor-I receptor defect associated with short stature and impaired carbohydrate homeostasis in an Italian pedigree. Horm Res Paediatr. 2011;76:136-143. Epub 2011 Jul 29
40. Kawashima Y, Higaki K, Fukushima T, Hakuno F, Nagaishi J, Hanaki K, Nanba E, Takahashi S, Kanzaki S. Novel missense mutation in the IGF-I receptor L2 domain results in intrauterine and postnatal growth retardation. Clin Endocrinol (Oxf). 2012;77:246-254.
41. Fujimoto M, Kawashima Sonoyama Y, Hamajima N, Hamajima T, Kumura Y, Miyahara N, Nishimura R, Adachi K, Nanba E, Hanaki K, Kanzaki S. Heterozygous nonsense mutations near the C-terminal region of IGF1R in two patients with small-for-gestational-age-related short stature. Clin Endocrinol (Oxf). 2015;83:834-841. Epub 2015 May 11

CASE REPORT

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A Boy with Reset Osmostat Who Developed Chronic Hyponatremia due to Hypothalamic Injury Caused By a Giant Arachnoid Cyst

Junko Naganuma, Satomi Koyama, Yoshiyuki Watabe, Shigemi Yoshihara

Dokkyo Medical University Faculty of Medicine, Department of Pediatrics, Tochigi, Japan

What is already known on this topic?

Reset osmostat (RO), a subtype of syndrome of inappropriate antidiuretic hormone (ADH) secretion, is a rare cause of hyponatremia characterized by a decrease in the plasma osmolality threshold for ADH excretion.

What this study adds?

For the first time, we report an 11-year-old boy diagnosed with RO and a giant arachnoid cyst in the prepontine cistern. Sodium correction was considered unnecessary for RO, but chronic hyponatremia must be treated for the risk of decreased bone density and growth obstacles.

Abstract

Reset osmostat (RO) is classified as type C among the four subtypes of the syndrome of inappropriate secretion of antidiuretic hormone based on antidiuretic hormone (ADH) secretion. It is characterized by a lower plasma osmolality threshold for ADH excretion when plasma sodium concentration is reduced. We report the case of a boy with RO and a giant arachnoid cyst (AC). The patient had been suspected of having AC since the fetal period, and a giant AC in the prepontine cistern was confirmed by brain magnetic resonance imaging seven days after birth. During the neonatal period, there were no abnormalities in the general condition or blood tests, and he was discharged from neonatal intensive care at 27 days after birth. He was born with a -2 standard deviation score birth length and mild mental retardation. When he was six years old, he was diagnosed with infectious impetigo and had hyponatremia of 121 mmol/L. Investigations revealed normal adrenal and thyroid functions, plasma hypo-osmolality, high urinary sodium, and high urinary osmolality. The 5% hypertonic saline and water load tests confirmed that ADH was secreted under low sodium and osmolality conditions, and the ability to concentrate urine and excrete a standard water load; therefore, RO was diagnosed. In addition, an anterior pituitary hormone secretion stimulation test was performed, which confirmed growth hormone secretion deficiency and gonadotropin hyperreactivity. Hyponatremia was untreated, but fluid restriction and salt loading were started at 12 years old because of the risk of growth obstacles. The diagnosis of RO is important from the viewpoint of clinical hyponatremia treatment options.

Keywords: Hyponatremia, reset osmostat, syndrome of inappropriate secretion of antidiuretic hormone, arachnoid cyst

Introduction

The human body has a mechanism for maintaining plasma osmotic pressure, volume, and composition homeostasis. Plasma osmotic pressure is primarily regulated by water intake due to thirst and urine volume via antidiuretic hormone (ADH) (1). Therefore, plasma sodium concentrations in healthy people are maintained within a narrow range of 135-145 mmol/L, despite wide variations in water and salt intake (2). In addition, plasma osmolality is closely regulated between 285 and 295 mOsm/kg via a complex interaction between ADH secretion and

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Address for Correspondence: Junko Naganuma MD, Dokkyo Medical University Faculty of Medicine, Department of Pediatrics, Tochigi, Japan
E-mail: i-junko@dokkyomed.ac.jp **ORCID:** orcid.org/0000-0003-0204-064X

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action and the sensation of thirst, which promotes water intake (2). The hypothalamus, which contributes to the osmotic control of thirst and salt appetite, partly mediates behavioral responses (3). The hypothalamus also regulates neuroendocrine responses by regulating the rate of renal sodium and water excretion through changes in the release of neurohypophysial natriuretic hormone and ADH (4).

Hyponatremia, a serum sodium level < 135 mmol/L, is the most common electrolyte disorder encountered in clinical practice (5). Hyponatremia has several etiologies and can be classified into hypotonic hyponatremia (further classified as hypovolemic, euvolemic, or hypervolemic), pseudohyponatremia, and non-hypotonic hyponatremia. Euvolemic hyponatremia is caused by an increase in the relative absolute volume of body water, and the syndrome of inappropriate antidiuresis (SIADH) is the most common cause of euvolemic hyponatremia in hospitalized patients (5). SIADH has a vast spectrum of etiologies and differential diagnoses and has classically been divided into four types (A, B, C, and D) (6). Type A is characterized by high erratic fluctuations in ADH that are not physiologically affected by plasma osmolality. Type B involves elevated basal secretion of vasopressin despite normal regulation by osmolality. Type C is a rare condition called reset osmostat (RO), which is characterized by a decrease in the plasma osmolality threshold for ADH excretion. Type D is characterized by normal osmoregulation of ADH; these cases relate to V2R receptor mutations that lead to constitutive activation of the receptor in the absence of ADH (7).

Type C, or RO, is infrequently encountered and poorly recognized. Unlike traditional SIADH, RO lowers the osmotic threshold for ADH release while maintaining the tubular volume of dilution and urine concentration (6). Herein, we report the case of an 11-year-old boy who had a giant arachnoid cyst (AC) that pressed on the hypothalamus in the prepontine cistern, and he was diagnosed with RO due to chronic hyponatremia.

Case Report

This was the case of an 11-year-old boy. During the 27th week of pregnancy, ACs were identified. The patient was born via cesarean section at 38 weeks and 3 days gestation, with a birth length of 49 cm and weight of 3.477 g. Apgar scores were 9 and 10 at 1 min and 5 min, respectively. At seven days after birth, a giant AC was confirmed in the prepontine cistern using brain magnetic resonance imaging (MRI). Respiratory and circulatory functions were maintained, and oral feeding was adequate. Blood tests revealed no abnormalities in electrolytes (serum sodium level was 138-142 mmol/L), blood glucose level, or blood count, and the patient was discharged on the 27th day after birth.

The AC tended to expand temporarily on MRI during infancy; however, there was no evidence of increased intracranial pressure, and the size of the AC did not change after about a year, and thus the progression was observed without surgery. He had a mild developmental delay in early childhood and had an intelligence quotient of 87 at five years of age. In addition, he grew with a short stature of about -2 standard deviations (SD). When he visited an outpatient clinic for infectious impetigo at 6 years and 1 month, a serum sodium level of 121 mmol/L was discovered, indicating hyponatremia. Initially, SIADH was suspected, and the patient was admitted to hospital with water restriction (15 mL/kg/day), 3% hypertonic saline load (7.4 mEq/kg/day), and administration of diuretics (1 mg/kg of intravenous furosemide). The serum sodium level temporarily increased to 134 mmol/L; however, it decreased to 125 mmol/L after treatment. His general condition was stable, and he was subsequently discharged from the hospital. One month later, he was re-examined for hyponatremia. During a physical examination at 6 years and 2 months of age, his height was 104.6 cm (-2.1 SD), weight was 16.6 kg, and body mass index was 15.2. He had clear consciousness and no skin swelling or edema. The blood pressure was 111/64 mmHg, pulse rate was 95 beats/min, temperature was 36.7 °C, and SpO_2 level was 98% (room air). The external genital Tanner classification was 1, and the testis volume was 1 cm^3 bilaterally. Red blood cell count was 3.94×10^4/μL, hemoglobin was 10.9 g/dL, hematocrit was 30.5%, white blood cell count was 6,500/mm^3, and platelets were 484,000/mm^3. The venous blood gas analysis was normal. Serum sodium was 127 mmol/L, serum potassium was 4.5 mmol/L, serum chlorine was 94 mmol/L, blood urea nitrogen was 14 mg/dL, uric acid was 2.1 mg/dL, creatinine was 0.19 mg/dL, and fasting blood glucose was 90 mg/dL. There were no abnormalities in hormones, including thyroid and adrenal hormones, with adrenocorticotropic hormone of 10.8 pg/mL, cortisol of 18 μg/dL, free triiodothyronine of 2.76 pg/mL, free thyroxine of 1.05 ng/dL and thyroid-stimulating hormone, 2.44 μIU/mL. However, the insulin-like growth factor was 32 ng/mL, which was low for his age. Testosterone was < 0.02 ng/mL, follicle-stimulating hormone basal value was 0.95 μIU/mL, and luteinizing hormone (LH) basal value was 0.60 IU/mL, which were pre-pubertal values. Urinalysis revealed a specific gravity of 1.028, pH of 7.0, glucosuria (-), and proteinuria (±). Urine biochemistry had a urinary sodium and creatine level of 170 mmol/L and 75 mg/dL, respectively.

When the brain MRI was re-examined and compared with the previous year, the size of the AC in the prepontine cistern, the degree of enlargement of the bilateral ventricles, and the findings of hypothalamus-pituitary stalk exclusion to the cranial side were unchanged (Figure 1).

When he complained of thirst, a biochemical blood test revealed that his serum sodium level was 125 mmol/L, and plasma osmolality was 266 mOsm/kg. Subsequently, a 5% hypertonic saline load test (infused over 120 min at a dose rate of 0.05 mL/kg/min) was performed. The values before and 60, 120, and 180 min after hypertonic saline loading were: serum sodium level, 127-132-134-135 mmol/L; plasma osmolality, 262-267-271-275 mOsm/kg; urine osmolality, 853-618-566-618 mOsm/kg; and ADH level, 2.4-1.8-2.1-14.1 pg/mL, respectively. This confirmed that low serum sodium and plasma osmolality increased ADH secretion.

Based on the results, we suspected type C SIADH and conducted a water load test (Figure 2). After drinking 350 mL (≈20 mL/kg) of water, 294 mL (84% of water intake) of urine was observed after 4 h, and diluted urine with a urine osmotic pressure of 61 mOsm/kg was confirmed. Furthermore, the fraction of urate excretion (FEUa) measured during the course of the test was 7.9%. From these results, the cause of chronic hyponatremia was diagnosed as RO.

In addition, the patient had a short stature and underwent an anterior pituitary hormone secretion stimulation test (Figure 3). He had no abnormalities in thyrotropin-releasing hormone or corticotropin-releasing hormone secretion. However, he was diagnosed with moderate growth hormone (GH) deficiency (GHD) because he had a peak GH value of 3.69 ng/mL on a clonidine load test. In addition, even before puberty, a LH-releasing hormone loading test confirmed a gonadotropin overreaction with LH as the predominant hormone.

After the examination, he was discharged from the hospital and followed-up at the outpatient department. Subsequently, the serum sodium level has remained around 120 mmol/L. There were occasional complaints of headache and vomiting but brain MRI revealed no evidence of the AC expanding. He has been on follow-up and has been encouraged to maintain mild fluid restriction and salt intake for his hyponatremia. GHD was initially followed-up with no hope of effective treatment, but his parents persisted. Thus, we commenced GH injections at 11 years and four months. At this age, the external genital Tanner stage was 1, and the testis volume was 1 cm^3 bilaterally, and physical findings revealed no signs of precocious puberty.

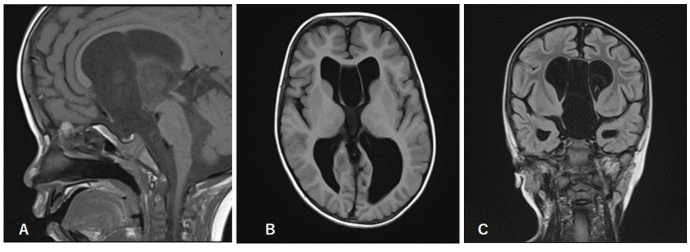

Figure 1. Brain magnetic resonance imaging. A) Sagittal section, T1W1; B) Coronal section, fluid attenuated ınversion recovery; C) Cross section, T1W1. The hypothalamus and pituitary stalk are anteriorly excluded by a giant arachnoid cyst in the prepontine cistern

Discussion

Hyponatremia (serum sodium level of less than 135 mmol/L) is caused by three mechanisms: inability to excrete water loads, excessive sodium loss, or inadequate sodium intake (8). As an algorithm for diagnosing hyponatremia, one should confirm whether it is isotonic, hypotonic, or

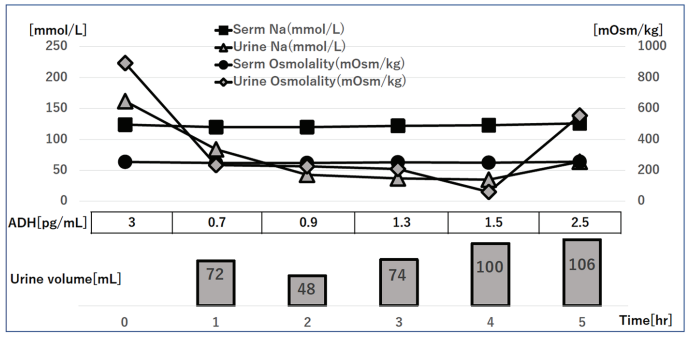

Figure 2. Effect of 350 mL (≈20 mL/kg) water load test. There was 294 mL (≈84%) of urine excretion in 4 h, and the urine osmolality was below 100 mOsm/kg

ADH: antidiuretic hormone

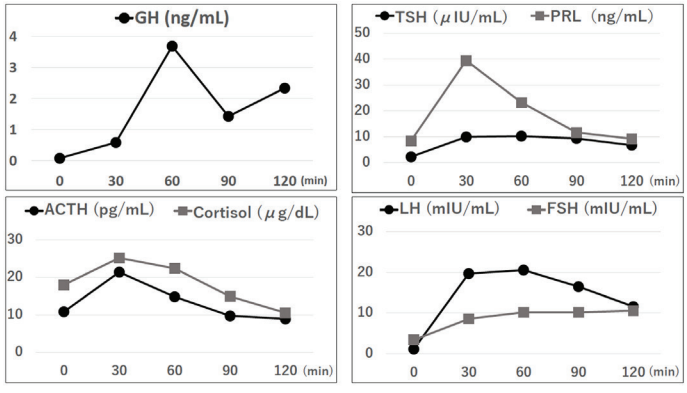

Figure 3. Clonidine/thyrotropin-releasing hormone/corticotropin-releasing hormone/luteinizing hormone-releasing hormone stimulation test. The results demonstrated growth hormone deficiency and gonadotropin overreaction

GH: growth hormone, TSH: thyroid-stimulating hormone, LH: luteinizing hormone, ACTH: adrenocorticotropic hormone, PRL: prolactin

hypertonic before proceeding with the differential diagnosis (8). Hypotonic hyponatremia is further classified as hypovolemia, euvolemia, or hypervolemia and should be differentiated according to the urinary sodium level of 20 mmol/L more or less (9). Hyponatremia is typically treated by suppressing ADH secretion, resulting in the excretion of maximally diluted urine (≤100 mOsm/kg). Most patients with hyponatremia and hypo-osmolarity are unable to retain water due to continuous ADH secretion, even though their urinary osmolality is above 100 mOsm/kg and density is above 1.003 (6). This euvolemic hyponatremia is the cause of 60% of all types of chronic hyponatremia, with SIADH being the most common cause, and other causes include hypothyroidism, glucocorticoid deficiency, and inadequate fluid therapy (6). The diagnostic criteria for SIADH described by Bartter and Schwartz (7) in 1967 are hyponatremia with plasma hypo-osmolarity, high urinary osmolality relative to plasma osmolality, increased urinary sodium excretion, absence of edema or volume depletion, and normal renal and adrenal functions (9). All of the criteria were met in the presented case.

The diagnostic criteria for RO include normovolemic hypotonic hyponatremia; normal renal, adrenal, and thyroid function; ability to concentrate urine; ability to excrete a standard water load, with excretion of more than 80% within four hours; maintenance of urine osmolality at or below 100 mOsm/kg during sustained water diuresis; and maintenance of normal sodium balance salt loading (10). The presented patient met these diagnostic criteria, and the water load test was crucial in diagnosing RO (11), with 84% of the loaded water content urinated within four hours. In addition, RO should exhibit a normal FEUa (4-11%) (12), which was noted in our patient (7.9%). First, a common cause of RO could be damage to the posterior pituitary (13). Changes in plasma osmotic pressure are caused by changes in the balance between the ingress and egress of electrolytes and water into the living body and are detected by osmotic receptors in the anterior hypothalamus (14). The osmotic receptors are present in the late paraventricular and subfornical organs of the anterior paraventricular nucleus of the third ventricle, and an increase in plasma osmotic pressure is detected at these sites, and the hypothalamus paraventricular nucleus and supraoptic nucleus. The signal is transmitted to the ADH neurons, and the posterior hypothalamus promotes ADH secretion (15).

Changes in osmolality are susceptible to changes in ADH secretion, and even a 1% increase in plasma osmolality increases ADH secretion (16). Moreover, there is an osmotic pressure threshold for ADH secretion, and ADH secretion is normally inhibited when the plasma osmotic pressure is about 280 mOsm/kg H_2O or less (16). When plasma osmolality exceeds this threshold, ADH secretion increases linearly (16). In addition, when the plasma osmotic pressure is approximately 290 mOsm/kg H_2O or higher, the thirst center is stimulated to induce drinking behavior, which contributes to the maintenance of body fluid volume as well as an increase in ADH secretion (16). Head trauma or intracranial lesions cause an abnormal resetting of these strict mechanisms of hypothalamic osmoreceptors, resulting in an inappropriate antidiuretic response to perceiving lower serum sodium levels. In case presented here, results consistent with RO findings in the hypertonic saline loading test were noted. The temporary decrease in ADH level, despite a mild increase in plasma osmolality, at 60 minutes after loading may have been due to the addition of water loading to the preload dehydration situation, in addition to the sodium loading.

Case reports of RO are rare, and pediatric reports are even rarer. The majority are also associated with median defects, such as cleft lip and palate, corpus callosum agenesis, pituitary disorder, and hypothalamic cysts (6,17,18,19). In the presented patient, the giant AC in the prepontine cistern displaced the anterior ventricular wall of the third ventricle, including the paraventricular nucleus of the hypothalamus, which is involved in ADH secretion.

Our case suggests that the cause of RO may be an abnormal reset of the hypothalamic osmoreceptor. The second possibility is sick cell syndrome. “Sick cells” have less effective osmotic pressure and thus their volume decreases, triggering vasopressin release. In severe hyponatremia, the release of ADH to retain water inside the cells is high; consequently, these cells begin to swell, exceeding their original size and inhibit lowering of serum sodium levels (20).

Previously, it was thought that correction of hyponatremia was unnecessary in RO because increased plasma sodium level and osmolality promote ADH secretion (21). However, recent evidence in adults suggests that chronic hyponatremia is associated with attention deficits (22), cognitive impairments (23), bone fractures, and osteoporosis (24). Hyponatremic rats showed decreased bone mineral density, in both trabecular and cortical bones, and significantly increased osteoclast activity (25). Although our patient presented with short stature, the GHD was moderate, as revealed by the GH secretion stimulation test results. Furthermore, no significant decrease was noted in the growth rate without GH treatment until the age of 11 years and 4 months. After initiation of GH treatment, his growth rate was increased slightly. In addition, bone mineral density performed by dual energy X-ray absorptiometry in the lumbar spine (L1-L4) was 0.470 g/cm^2 at 12 years

of age, with an age-matched Z-score of -5.1. Thus, we also considered the possibility that the effect of chronic hyponatremia, in addition to GHD, on bone resulted in short stature. In a report of an 8-year-old girl with a hypothalamic glioma complicated by RO who had difficulty with fluid restriction, oral administration of tolvaptan, an arginine vasopressin V2 receptor antagonist, showed improvement in hyponatremia and relief of fluid restriction (19). At present, data on the use of tolvaptan in children are limited, and caution must be exercised during long-term use, especially with regard to the side effect of liver dysfunction due to hepatotoxicity. However, there is also a report that suggests that oral tolvaptan was safely administered to three pediatric patients with chronic hyponatremia due to SIADH (26).

To the best of our knowledge, the present study is the only case report of a child with severe hyponatremia caused by RO due to an AC. This patient was initially thought to have no need for treatment to correct hyponatremia due to RO. However, to avoid adverse events caused by chronic hyponatremia, which have become evident in recent years, it is necessary to restrict salt and excessive fluid intake and to continue careful monitoring of the patient's progress, while considering tolvaptan administration.

Conclusion

In asymptomatic patients with severe hyponatremia, RO should be considered. RO is uncommon and very rare in children. In this present case, RO may have been induced by physical pressure on the hypothalamus or by a disability. Further case series and studies are warranted to define the need for treatment of chronic hyponatremia caused by RO in children.

Ethics

Informed Consent: Informed consent was obtained from the parent of the patient for publication of this case.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Junko Naganuma, Satomi Koyama, Yoshiyuki Watabe, Concept: Junko Naganuma, Design: Junko Naganuma, Data Collection or Processing: Junko Naganuma, Satomi Koyama, Analysis or Interpretation: Junko Naganuma, Satomi Koyama, Yoshiyuki Watabe, Shigemi Yoshihara, Literature Search: Junko Naganuma, Writing: Junko Naganuma.

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References

1. Robertson GL, Mahr EA, Athar S, Sinha T. Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J Clin Invest. 1973;52:2340-2352.
2. Reynolds RM, Padfield PL, Seckl JR. Disorders of sodium balance. BMJ. 2006;332:702-705.
3. Upadhyay A, Jaber BL, Madias NE. Epidemiology of hyponatremia. Semin Nephrol. 2009;29:227-238.
4. Sharif-Naeini R, Ciura S, Zhang Z, Bourque CW. Contribution of TRPV channels to osmosensory transduction, thirst, and vasopressin release. Kidney Int. 2008;73:811-815. Epub 2008 Jan 16
5. Dineen R, Thompson CJ, Sherlock M. Hyponatraemia - presentations and management. Clin Med (Lond). 2017;17:263-269.
6. Vale BM, Morais S, Mesquita J, Mimoso G. Reset osmostat: a rare cause of hyponatraemia. BMJ Case Rep. 2015;2015:bcr201300911.
7. Bartter FC, Schwartz WB. The syndrome of inappropriate secretion of antidiuretic hormone. Am J Med. 1967;42:790-806.
8. Kuthiah N, Er C. Reset Osmostat: A Challenging Case of Hyponatremia. Case Rep Med. 2018;2018:5670671.
9. Braun MM, Barstow CH, Pyzocha NJ. Diagnosis and management of sodium disorders: hyponatremia and hypernatremia. Am Fam Physician. 2015;91:299-307.
10. Feder J, Gomez JM, Serra-Aguirre F, Musso CG. Reset Osmostat: Facts and Controversies. Indian J Nephrol. 2019;29:232-234.
11. Robertson GL. Regulation of arginine vasopressin in the syndrome of inappropriate antidiuresis. Am J Med. 2006;119(7 Suppl 1):36-42.
12. Bardanzellu F, Marcialis MA, Frassetto R, Melis A, Fanos V. Differential diagnosis between syndrome of inappropriate antidiuretic hormone secretion and cerebral/renal salt wasting syndrome in children over 1 year: proposal for a simple algorithm. Pediatr Nephrol. 2022;37:1469-1478. Epub 2021 Sep 1
13. Imbriano LJ, Mattana J, Drakakis J, Maesaka JK. Identifying Different Causes of Hyponatremia With Fractional Excretion of Uric Acid. Am J Med Sci. 2016;352:385-390. Epub 2016 Jul 6.
14. Denton DA, McKinley MJ, Weisinger RS. Hypothalamic integration of body fluid regulation. Proc Natl Acad Sci U S A. 1996;93:7397-7404.
15. Watanabe E, Fujikawa A, Matsunaga H, Yasoshima Y, Sako N, Yamamoto T, Saegusa C, Noda M. Nav2/NaG channel is involved in control of salt-intake behavior in the CNS. J Neurosci. 2000;15;20:7743-7751.
16. Robertson GL, Shelton RL, Athar S. The osmoregulation of vasopressin Kidney Int. 1976;10:25-37.
17. Thiagarajan R, La Gamma E, Dey S, Blethen S, Wilson TA. Hyponatremia caused by a reset osmostat in a neonate with cleft lip and palate and panhypopituitarism. J Pediatr. 1996;128:561-563.
18. Gupta P, Mick G, Fong CT, Jospe N, McCormick K. Hyponatremia secondary to reset osmostat in a child with a central nervous system midline defect and a chromosomal abnormality. J Pediatr Endocrinol Metab. 2000;13:1637-1641.
19. Krishnan S, Deshpande S, Mallappa A, Gunda D, Lane P, Vishwanath A, McNall-Knapp RY. Corrigendum to "Management Challenges in a Child with Chronic Hyponatremia: Use of V2 Receptor Antagonist". Case Rep Pediatr. 2017;2017:8132129. Epub 2017 Jul 2 Erratum for: Case Rep Pediatr. 2017;2017:3757423.
20. Benson M, Canas JA. Chronic hyponatremia and failure to thrive in an 18 month old toddler with chronic SIADH. Endotrends 2010;17:11-13. Available from: https://l24.im/uN94Fpf
21. Lai YY, Samsudin IN, Mohamed Mokhtar N, C Thambiah S. Persistent Hyponatremia in an Elderly Patient. Clin Chem. 2020;66:652-657.

22. Renneboog B, Musch W, Vandemergel X, Manto MU, Decaux G. Mild chronic hyponatremia is associated with falls, unsteadiness, and attention deficits. Am J Med. 2006;119:1-8.

23. Gunathilake R, Oldmeadow C, McEvoy M, Kelly B, Inder K, Schofield P, Attia J. Mild hyponatremia is associated with impaired cognition and falls in community-dwelling older persons. J Am Geriatr Soc. 2013;61:1838-1839.

24. Murthy K, Ondrey GJ, Malkani N, Raman G, Hodge MB, Marcantonio AJ, Verbalis JG. The effects of hyponatremia on bone density and fractures: A systematic review and meta-analysis. Endocr Pract. 2019;25:366-378. Epub 2019 Jan 18

25. Verbalis JG, Barsony J, Sugimura Y, Tian Y, Adams DJ, Carter EA, Resnick HE. Hyponatremia-induced osteoporosis. J Bone Miner Res. 2010;25:554-563.

26. Tuli G, Tessaris D, Einaudi S, De Sanctis L, Matarazzo P. Tolvaptan Treatment in Children with Chronic Hyponatremia due to Inappropriate Antidiuretic Hormone Secretion: A Report of Three Cases. J Clin Res Pediatr Endocrinol. 2017;9:288-292. Epub 2017 May 17

CASE REPORT

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Seminoma in 46, XY Gonadal Dysgenesis: Rare Presentation and Review of the Literature

Maamoun Adra[1,2], Hayato Nakanishi[1,2], Eleni Papachristodoulou[3], Evangelia Karaoli[3], Petroula Gerasimou[4], Antri Miltiadous[4], Katerina Nicolaou[4], Loizos Loizou[3], Nicos Skordis[1,2,5]

[1]St George's University of London Medical School, London, UK
[2]University of Nicosia Medical School, Nicosia, Cyprus
[3]Makarios Hospital, Clinic of Paediatric Oncology, Nicosia, Cyprus
[4]Karaiskakio Foundation, Molecular Hematology-Oncology, Nicosia, Cyprus
[5]Private Practice, Division of Pediatric Endocrinology, Nicosia, Cyprus

What is already known on this topic?

Swyer syndrome is a rare congenital condition that is known to be a risk factor for developing germ cell tumors. 46, XY gonadal dysgenesis (GD) involves a high risk of gonadoblastoma development with malignant potential such that the onset is greatest at or after the event of puberty.

What this study adds?

This study reports a 12-year-old phenotypic female with 46, XY GD, who developed an advanced metastatic seminoma. Furthermore, a review of the literature was performed in order to highlight the rarity of the development of seminoma in the context of 46, XY complete GD.

Abstract

Swyer syndrome is a rare congenital condition that serves as a risk factor for developing germ cell tumors. The condition belongs to the group of 46, XY disorders of sexual development, is characterized by complete gonadal dysgenesis (CGD) and is mostly manifested as delayed puberty and primary amenorrhea during adolescence. Individuals with Swyer syndrome are known to be phenotypically female with normal internal and external female genitalia at birth. 46, XY GD involves a high risk of gonadoblastoma development with malignant potential such that the onset is greatest at or after the event of puberty. This report of a 12-year-old phenotypic female with 46, XY GD, who developed an advanced metastatic seminoma, highlights the rarity of the development of a seminoma in the context of 46, XY CGD.

Keywords: Seminoma, Swyer syndrome, gonadal dysgenesis, 46, XY

Introduction

Disorders of sexual differentiation (DSD) are congenital conditions such that the chromosomal profile, gonadal sex, and phenotypic appearance of the external genitalia of the individual are discordant (1). The broad categories that fall under DSD include 46 XY DSD, 46 XX DSD, ovotesticular DSD, and sex chromosome DSD, for example 47 XXY, 45 X, and 45 X/46 XY. Individuals with 46, XY DSD present with a varied clinical picture, from females with normal external genitalia to under-virilized males (2).

The underlying cause of 46, XY DSD involve gonadal dysgenesis (GD) or dysfunction of the synthesis or action of androgens or anti-Müllerian hormone (AMH) (3). GD,

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Address for Correspondence: Nicos Skordis MD, St George's University of London Medical School, London, UK; University of Nicosia Medical School; Private Practice, Division of Pediatric Endocrinology, Nicosia, Cyprus
E-mail: nicosskordis@paedi.org.cy **ORCID:** orcid.org/0000-0002-0338-8317

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previously known as sex reversal (4), can manifest as complete GD (CGD) or partial GD. Disorders of androgen synthesis or action involve various enzymatic defects of testosterone synthesis or conversion and defective androgen receptors such as 17-beta HSD deficiency, 5α-reductase deficiency, and androgen insensitivity syndromes (1,5).

Swyer syndrome, first described by Swyer (6) in 1955, is a type of 46, XY DSD characterized by CGD. The incidence of all cases with 46, XY DSD in general is estimated to be 1:20,000. The incidence of the Swyer syndrome has been estimated to be 1:100,000 (1). The underlying pathology of this condition involves the presence of bilateral, non-functional streak gonads that fail to secrete testosterone and AMH. Therefore, individuals with Swyer syndrome are known to be phenotypically female with normal internal and external female genitalia at birth. The condition most commonly is manifested during adolescence as delayed puberty and primary amenorrhea (7).

Patients with 46, XY CGD have the highest tumor rate among a population of patients with DSD and the presence of a Y chromosome (8). Swyer syndrome involves a high risk of gonadoblastoma development with malignant potential, such that the onset is greatest at or after the event of puberty (9). Gonadoblastomas are *in situ* benign tumors that can transform to malignant germ cell tumors, such as dysgerminoma or seminoma (10). The risk of malignancy is reported as 37% to 45% (11,12). Once the diagnosis of Swyer syndrome is made, the patient should undergo a gonadectomy to prevent the development of gonadal malignancies (13). Furthermore, puberty is induced via estrogen supplementation for the development of secondary sexual characteristics. In the long term, hormone replacement therapy, including estrogen and progesterone, is given to maintain the menstrual cycle (1).

Swyer syndrome is a rare congenital condition that serves as a risk factor for developing germ cell tumors. This report of a 12-year-old, phenotypic female with 46, XY GD, who developed an advanced metastatic seminoma highlights the rarity of the development of a seminoma in the context of 46, XY CGD.

Case Report

A 12-year-old Caucasian phenotypic female with an apparently unremarkable past medical history presented with primary amenorrhea and a large abdominal mass. The patient comes from a non-consanguineous family and both parents and siblings are healthy and have no remarkable past medical history.

One month prior to her admission, the patient's mother noticed a palpable solid mass in the femoral-inguinal region. This gradually increasing palpable abdominal mass was accompanied by pain, abdominal distention, and constipation. The abdominal ultrasound imaging showed an abdominal mass and secondary liver metastasis.

Upon physical examination, her Tanner stage for breast and pubic hair was T1 and T2 respectively. She had palpable lymph nodes at the cervical and right axillary area. Her abdomen was distended, with a palpable and painful mass at the umbilical and left lateral region, with hepatomegaly. She also had a solid palpable mass at the left inguinal region, approximately 4 cm in size, and a smaller one at the right side. Computed tomography of the abdomen revealed a large occupying lesion with patchy inhomogeneous areas and multiple calcifications with diameter of 15x12x8.5 cm. Furthermore, there were blocks of multiple retroperitoneal sizeable masses involving the para-aortic areas, the celiac axis, the liver and bilateral renal hilar areas.

A tumor biopsy was performed, and the findings were morphologically and immunohistochemically compatible with a seminoma.

The patient's seminoma was treated according to the Testicular Cancer Protocol 2011 (14). She completed four cycles of the chemotherapy regimen PEB (bleomycin, etoposide, and cisplatin). After completion of chemotherapy, she underwent bilateral gonadectomy and was given hormonal treatment with estrogens for feminization and later for induction of menarche. She is currently on hormonal replacement therapy with combined estradiol and dydrogesterone. Her growth increased from 150 cm at 13 years to 166 cm at 17 years.

Lab Findings

Hormonal Findings

Pre-operative assessment of gonadal function was performed and the laboratory values demonstrated elevated gonadotrophins (follicle-stimulating hormone 76.25 UI/L, luteinizing hormone 16.08 UI/L), testosterone < 0.02 ng/mL, AMH < 1 pmol/L, inhibin B 10 pg/mL (normal: 10-200 pg/mL) and estradiol levels were < 10 pg/mL.

Genetic analysis was done using whole exome sequencing to check for any mutations that may be associated with the patient's phenotype. A family trio exome analysis (Agilent

exome V8) was employed for better extrapolation of results and possible candidate variants. No clinically relevant variants were detected in the genes tested, but there may be a pathogenic variant or deep intronic modifying mutations outside of the genetic regions of analysis. However, there were two interesting findings involving two genes: *ZNF133* and *COL4A1*. Even though the variations in these two genes are of *de novo* origin, their involvement in the patient's presentation remains unknown. The *ZNF133* gene encodes the Zinc finger protein 133, which is predicted to enable DNA-binding transcription repressor activity and be involved in negative regulation of transcription by RNA polymerase II, along with other functions (15). *COL4A1*, also known as collagen type 4 alpha 1 chain, is a gene found on chromosome 13, and is involved with the formation of the alpha 1 chain of type 4 collagen. This chain is part of a complex protein network that plays numerous roles in the body, such as helping the basement membranes interact with proximal cells, cellular migration, and cellular proliferation (16).

Karyotypic analysis (Agilent) of the bone marrow and peripheral blood revealed the patient to be 46, XY. In addition, fluorescence *in situ* hybridization analysis for *SRY* (sex-determining region Y) was performed revealing a signal pattern of one *SRY* signal and one DYZI, a human Y chromosome specific repeated DNA family, signal in all cells examined.

Tumor markers were investigated prior to the initiation of chemotherapy treatment. The patient had elevated levels of NSE (131.7 ng/mL; reference range: <16.3 ng/mL), CA 125 (209.4 U/mL; reference range: <35 U/mL), and β-hCG (79.33 U/L; reference range: <5 U/L) prior to the initiation of chemotherapy treatment.

Discussion

Swyer syndrome is synonymous with CGD in patients with an XY karyotype. The most widely accepted pathogenic mechanism in this condition is a mutation in the *SRY* gene, which is expressed in the germ cells and Sertoli cells. This gene is known to be responsible for converting the undifferentiated gonads into testes. The mutation leads to the production of a defective protein that does not permit the undifferentiated gonad to develop, resulting in the presence of streak gonads that fail to secrete testosterone and AMH (17). It is estimated that 15% of patients with 46, XY CGD have a mutation in the *SRY* gene (1). Patients with Swyer syndrome typically present with a female phenotype with normal external genitalia and Müllerian structures at birth and usually seek medical care in adolescence for delayed puberty with primary amenorrhea due to the lack of hormonal production by the gonads (7).

Since dysgenetic gonads have a 30% risk of developing a gonadoblastoma, the delayed nature of the diagnosis often results in patients already having developed a germ cell tumor at the time of prophylactic bilateral gonadectomy. A case series of three patients demonstrated the presence of gonadoblastoma in one patient and dysgerminoma in the two other patients incidentally at the time of gonadectomy (18).

Patients with Swyer syndrome are known to be at high risk of developing a germ cell tumor, the commonest example being a gonadoblastoma because they have Y chromosome material in their genome. The risk of developing a germ cell neoplasia in these patients depends on the presence of a region on the Y-chromosome known as the gonadoblastoma (GBY) region (19). Despite being benign tumors, gonadoblastomas have the potential to transform to malignant germ cell tumors in 50% to 60% of cases. Dysgerminomas are reported to be present in 22-66% of the cases (12). A recent study from Latvia demonstrated that gonadoblastomas and dysgerminomas were the most commonly diagnosed tumors in patients with Swyer syndrome and the authors stressed the importance of early diagnosis (20). Furthermore, malignant transformation to seminoma has been reported (21).

Testicular cancer is generally an uncommon type of cancer, forming only 1-2% of all tumors in men. However, it is the most common type of neoplasia among young men (22). Germ cell tumors are the most common type of testicular cancer, such that the occurrence of a seminoma versus a non-seminoma is approximately the same. Risk factors associated with testicular cancer usually arise in patients with undescended testes, a history of testicular cancer, a family history of testicular cancer or GD (22).

The patient reported herein with GD presented with a very rare gonadal tumor.

The development of seminoma in patients with 46, XY GD is very rare with only two previously confirmed reported cases (Table 1). The first case was an 18-year-old female who presented with primary amenorrhea and pelvic masses. She had a seminoma on her right gonad, which was confirmed to be an ovotestis (23). The second case was a 16-year-old female who presented with primary amenorrhea. She had a seminoma on her left gonad, which was confirmed to be

Table 1. The type of tumor reported in patients with Swyer syndrome

Study	Tumor type	Patient age
Alam et al. (9), 2020	Gonadoblastoma/ dysgerminoma	16 years old
Anwar et al. (25), 2021	Dysgerminoma	16 years old
Arafa et al. (26), 2021	Gonadoblastoma/ dysgerminoma	17 years old
Behtash and Karimi Zarchi (27) 2007	Dysgerminoma	20, 19, 17 years old
Ben Temime et al. (28), 2008	Gonadoblastoma/ dysgerminoma	13 years old
Bjersing and Kjellgren (29) 1977	Dysgerminoma or seminoma (not confirmed)	18 years old
Bumbulienė et al. (7), 2020	Gonadoblastoma/ dysgerminoma	9 years old
Çatlı et al. (30), 2015	Gonadoblastoma	15 years old
Chen et al. (23), 2015	Seminoma	18 years old
Dural et al. (31), 2019	Gonadoblastoma/ dysgerminoma	17 years old
Gonzalez-Benitez et al. (32), 2015	Gonadoblastoma/ dysgerminoma	29 years old
Hamed and Hanafy (33) 2021	Gonadoblastoma/ dysgerminoma	19 years old
Han et al. (34), 2011	Dysgerminoma	21 years old
Hanlon and Kimble (18) 2015	Gonadoblastoma/ dysgerminoma	17, 15, 15 years old
Ilter et al. (35), 2008	Gonadoblastoma/ dysgerminoma	26 years old
Jadhav et al. (36), 2006	Gonadoblastoma/ dysgerminoma	19 years old
Jonson et al. (21), 2010	Gonadoblastoma/ dysgerminoma, seminoma	20, 16 years old
Kim et al. (37), 1993	Gonadoblastoma/ dysgerminoma	24 years old
Kumar et al. (38), 2016	Teratoma/dysgerminoma/ yolk sac	14 years old
Milewicz et al. (39), 2016	Gonadoblastoma/ dysgerminoma	18 years old
Stachowicz-Stencel et al. (40), 2011	Choriocarcinoma/ dysgerminoma	14 years old
Yada-Hashimoto et al. (41), 2018	Dysgerminoma	25 years old
Zhu et al. (42), 2011	Dysgerminoma	22 years old
Zhu et al. (43), 2016	Teratoma/dysgerminoma/ yolk sac tumor	16 years old

a streak gonad (21). Our patient would be the third case of seminoma in Swyer syndrome presenting with a palpable pelvic mass to be reported, to the best of our knowledge. A recent cross-sectional multicenter study in 1,040 patients with DSD above the age of 16 years of whom 21 patients had CGD showed that those with CGD had the highest risk (33%) of developing a germ cell neoplasia (24). Among those patients reported in the study, only one patient with CGD had a seminoma.

The work of Elzaiat et al. (3), 2022 demonstrated the most recent genetic basis of 46, XY GD. There seems to be an extensive list of genes and proposed candidates that are associated with the 46, XY GD phenotype, including *SRY*, *SOX9*, *DMRT1*, and *DHH*. *SRY* activates the expression of *SOX9*, a downstream effector that's responsible for Sertoli cells formation. Testis development control can be achieved by the close binding between DMRT1 and SOX9 on target genes in the fetal testis (3). DHH is a protein expressed by Sertoli cells that is responsible for genetic regulation in Leydig cells as well as peritubular myoid cells (2). Figure 1 illustrates a simplified schematic of how the different genes interact. Mutations in this molecular pathway have been shown to be associated with 46, XY CGD (3). In the presented patient, current genetic testing failed to identify the genetic defect associated with her condition, highlighting the need for further experimentation. In the future technological advances in molecular methodologies, such as whole genome sequencing, optical genome mapping and next-generation cytogenetics may aid in elucidating pathogenesis and prognosis of this disorder.

Conclusion

Due to a scarcity of reported cases, we present a very rare case of a patient with Swyer syndrome who developed an advanced metastatic seminoma. Despite the metastatic nature of the seminoma and the symptomatic presentation of the patient, she achieved a good overall outcome after undergoing chemotherapy, bilateral gonadectomy and feminization therapy. This case report and the limited literature included in this review highlight the rarity of a seminoma and the importance of early detection of Swyer syndrome with a subsequent earlier prophylactic bilateral gonadectomy and meticulous follow-up to prevent the development of gonadal malignancy in this group of patients.

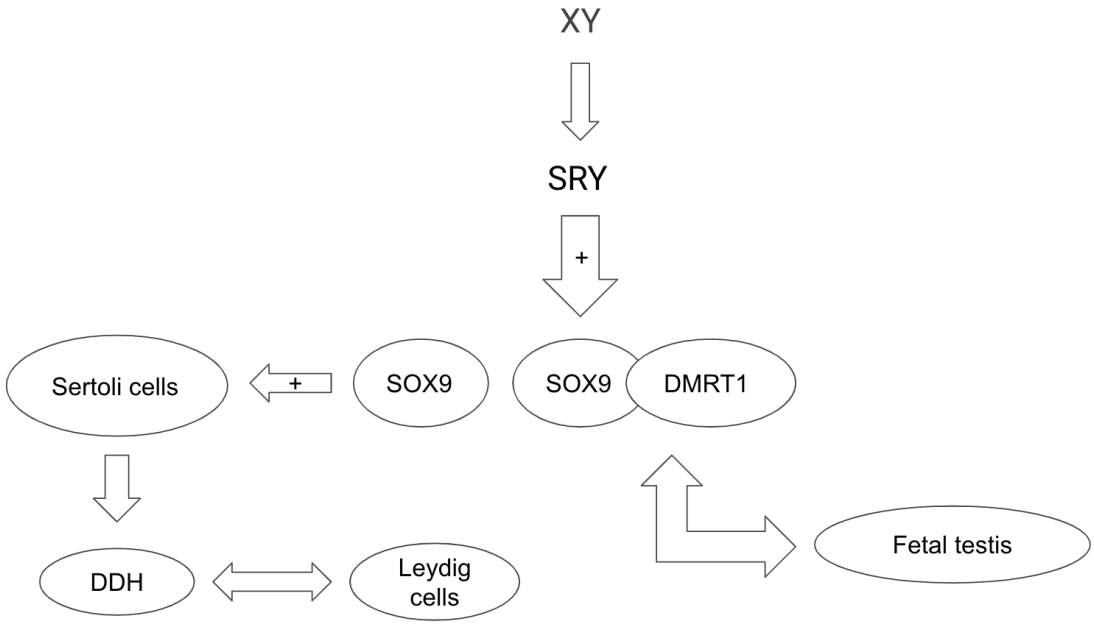

Figure 1. Simplified pathway of the genes regulating testicular differentiation

The *SRY* gene (found on Yp11.3) activates the expression of *SOX9* (found on 17q24.3), which is responsible for the formation of Sertoli cells and regulation of testis development via close binding with DMRT1.

DMRT1 (found on 9p24.3) regulates testis development via close binding with SOX9.

DDH (12q13.12) is responsible for development of the Leydig cells during fetal life.

Ethics

Informed Consent: Informed consent was obtained from those included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Antri Miltiadous, Evangelia Karaoli, Eleni Papachristodoulou, Katerina Nicolaou, Loizos Loizou, Nicos Skordis, Petroula Gerasimou, Concept: Hayato Nakanishi, Maamoun Adra, Nicos Skordis, Petroula Gerasimou, Design: Hayato Nakanishi, Maamoun Adra, Nicos Skordis, Petroula Gerasimou, Data Collection or Processing: Evangelia Karaoli, Eleni Papachristodoulou, Hayato Nakanishi, Loizos Loizou, Maamoun Adra, Nicos Skordis, Literature Search: Hayato Nakanishi, Maamoun Adra, Nicos Skordis, Writing: Hayato Nakanishi, Maamoun Adra, Nicos Skordis, Petroula Gerasimou.

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References

1. Bashamboo A, McElreavey K. Mechanism of Sex Determination in Humans: Insights from Disorders of Sex Development. Sex Dev. 2016;10:313-325. Epub 2016 Dec 3
2. Neocleous V, Fanis P, Cinarli F, Kokotsis V, Oulas A, Toumba M, Spyrou GM, Phylactou LA, Skordis N. 46,XY complete gonadal dysgenesis in a familial case with a rare mutation in the desert hedgehog (DHH) gene. Hormones (Athens). 2019;18:315-320. Epub 2019 Jun 25
3. Elzaiat M, McElreavey K, Bashamboo A. Genetics of 46,XY gonadal dysgenesis. Best Pract Res Clin Endocrinol Metab. 2022;36:101633. Epub 2022 Feb 25
4. Hughes IA, Houk C, Ahmed SF, Lee PA; LWPES Consensus Group; ESPE Consensus Group. Consensus statement on management of intersex disorders. Arch Dis Child. 2006;91:554-563. Epub 2006 Apr 19
5. Galli-Tsinopoulou A, Serbis A, Kotanidou EP, Litou E, Dokousli V, Mouzaki K, Fanis P, Neocleous V, Skordis N. 46,XY Disorder of Sex Development due to 17-Beta Hydroxysteroid Dehydrogenase Type 3 Deficiency in an Infant of Greek Origin. J Clin Res Pediatr Endocrinol. 2018;10:74-78. Epub 2017 Jul 24
6. Swyer GI. Male pseudohermaphroditism: a hitherto undescribed form. Br Med J. 1955;2:709-712.
7. Bumbulienė Ž, Varytė G, Geimanaitė L. Dysgerminoma in a Prepubertal Girl with Complete 46XY Gonadal Dysgenesis: Case Report and Review of the Literature. J Pediatr Adolesc Gynecol. 2020;33:599-601. Epub 2020 May 5
8. Lu L, Luo F, Wang X. Gonadal tumor risk in pediatric and adolescent phenotypic females with disorders of sex development and Y chromosomal constitution with different genetic etiologies. Front Pediatr. 2022;10:856128.
9. Alam S, Boro H, Goyal A, Khadgawat R. 46, XY complete gonadal dysgenesis with pubertal virilisation due to dysgerminoma/gonadoblastoma. BMJ Case Rep. 2020;13:e235501.
10. Piazza MJ, Urbanetz AA. Germ Cell Tumors in Dysgenetic Gonads. Clinics (Sao Paulo). 2019;74:e408.

11. de la Calle CM, Kim S, Baskin LS. Diagnosis and treatment of the intra-abdominal gonad in the pediatric population: Testes, ovaries, dysgenetic gonads, streaks, and ovotestes. J Pediatr Surg. 2020;55:2480-2491. Epub 2020 Feb 19
12. McCann-Crosby B, Mansouri R, Dietrich JE, McCullough LB, Sutton VR, Austin EG, Schlomer B, Roth DR, Karaviti L, Gunn S, Hicks MJ, Macias CG. State of the art review in gonadal dysgenesis: challenges in diagnosis and management. Int J Pediatr Endocrinol. 2014;2014:4. Epub 2014 Apr 14
13. Fallat ME, Donahoe PK. Intersex genetic anomalies with malignant potential. Curr Opin Pediatr. 2006;18:305-311.
14. Albers P, Albrecht W, Algaba F, Bokemeyer C, Cohn-Cedermark G, Fizazi K, Horwich A, Laguna MP; European Association of Urology. EAU guidelines on testicular cancer: 2011 update. Eur Urol. 2011;60:304-319. Epub 2011 May 25
15. Jung ES, Choi KW, Kim SW, Hübenthal M, Mucha S, Park J, Park Z, Ellinghaus D, Schreiber S, Franke A, Oh WY, Cheon JH. ZNF133 is associated with infliximab responsiveness in patients with inflammatory bowel diseases. J Gastroenterol Hepatol. 2019;34:1727-1735. Epub 2019 Apr 1
16. Vahedi K, Alamowitch S. Clinical spectrum of type IV collagen (COL4A1) mutations: a novel genetic multisystem disease. Curr Opin Neurol. 2011;24:63-68.
17. Jäger RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. Nature. 1990;348:452-454.
18. Hanlon AJ, Kimble RM. Incidental gonadal tumors at the time of gonadectomy in women with Swyer syndrome: a case series. J Pediatr Adolesc Gynecol. 2015;28:27-29. Epub 2014 Aug 12
19. Page DC. Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads. Development. 1987;101(Suppl):151-155.
20. Jakovleva A, Kovalova Z. Complete gonadal dysgenesis analysis in the population of Latvia: malignant outcomes and a review of literature. Med Pharm Rep. 2022;95:47-53. Epub 2022 Jan 31
21. Jonson AL, Geller MA, Dickson EL. Gonadal dysgenesis and gynecologic cancer. Obstet Gynecol. 2010;116(Suppl 2):550-552.
22. Manecksha RP, Fitzpatrick JM. Epidemiology of testicular cancer. BJU Int. 2009;104:1329-1333.
23. Chen Z, Wei Z, Zhao W, Wang J, Shen G, He C, Chen Y. Swyer syndrome with seminoma: laparoscopic bilateral gonad resection. J Minim Invasive Gynecol. 2015;22:532-533. Epub 2014 Nov 6
24. Slowikowska-Hilczer J, Szarras-Czapnik M, Duranteau L, Rapp M, Walczak-Jedrzejowska R, Marchlewska K, Oszukowska E, Nordenstrom A; dsd-LIFE group. Risk of gonadal neoplasia in patients with disorders/differences of sex development. Cancer Epidemiol. 2020;69:101800. Epub 2020 Sep 6
25. Anwar A, Akhtar M, Busby G. Swyer Syndrome: A Case of Dysgerminoma Solely within the Fallopian Tube. J Pediatr Adolesc Gynecol. 2021;34:869-871. Epub 2021 May 11
26. Arafa M, Ryiami MA, Shukri MA, Burney I, Mahfouz Y, Al-Kindi N. Bilateral Gonadoblastoma Overgrown by Dysgerminoma of the Right Gonad in a Patient with Swyer Syndrome. Maedica (Bucur). 2021;16:734-737.
27. Behtash N, Karimi Zarchi M. Dysgerminoma in three patients with Swyer syndrome. World J Surg Oncol. 2007;5:71.
28. Ben Temime R, Chachial A, Attial L, Ghodbanel I, Makhloufl T, Koubaal A, Kourda N, Ben Jilani S, Dammak T, El May A, Rahal K. 46 XY pure gonadal dysgenesis with gonadoblastoma and dysgerminoma. Tunis Med. 2008;86:710-713.
29. Bjersing L, Kjellgren O. Dysgerminomas (seminomas) in genetic males with female phenotype. One case of gonadal dysgenesis and gonadoblastoma and one of testicular feminization. Acta Obstet Gynecol Scand Suppl. 1977;66:27-37.
30. Çatlı G, Alparslan C, Can PŞ, Akbay S, Kelekçi S, Atik T, Özyılmaz B, Dündar BN. An Unusual Presentation of 46,XY Pure Gonadal Dysgenesis: Spontaneous Breast Development and Menstruation. J Clin Res Pediatr Endocrinol. 2015;7:159-162.
31. Dural O, Evruke I, Can S, Yasa C, Ugurlucan FG, Akhan SE. Atypical Presentation of Swyer Syndrome. J Pediatr Adolesc Gynecol. 2019;32:645-647. Epub 2019 Jul 26
32. Gonzalez-Benitez C, De La Iglesia E, De Santiago J, Zapardiel I. Dysgerminoma on a gonadoblastoma in a patient with Swyer syndrome treated with single incision laparoscopic surgery. J Obstet Gynaecol. 2015;35:102-103. Epub 2014 Jun 24
33. Hamed ST, Hanafy MAM. Swyer syndrome with malignant germ cell tumor: a case report. Egypt J Radiol Nucl Med. 2021;52:239.
34. Han Y, Wang Y, Li Q, Dai S, He A, Wang E. Dysgerminoma in a case of 46, XY pure gonadal dysgenesis (Swyer syndrome): a case report. Diagn Pathol. 2011;6:84.
35. Ilter E, Haliloğlu B, Akin FT, Karaman A, Ozden S. Pure 46,XY gonadal dysgenesis (Swyer syndrome) with breast development and secondary amenorrhea. Gynecol Obstet Invest. 2008;66:214-216. Epub 2008 Jul 22
36. Jadhav MN, Yelikar BR, Karigoudar M. Gonadoblastoma with contralateral dysgerminoma in a young female--a case report. Indian J Pathol Microbiol. 2006;49:274-276.
37. Kim SK, Sohn IS, Kim JW, Song CH, Park CI, Lee MS, Kim GW, Kim KR. Gonadoblastoma and dysgerminoma associated with 46,XY pure gonadal dysgenesis--a case report. J Korean Med Sci. 1993;8:380-384.
38. Kumar NP, M V, Mathews A, James FV. Mixed Germ Cell Tumour in a Case of Pure Gonadal Dysgenesis (Swyer Syndrome) - A Case Report. Cureus. 2016;8:459.
39. Milewicz T, Mrozińska S, Szczepański W, Białas M, Kiałka M, Doroszewska K, Kabzińska-Turek M, Wojtyś A, Ludwin A, Chmura Ł. Dysgerminoma and gonadoblastoma in the course of Swyer syndrome. Pol J Pathol. 2016;67:411-414.
40. Stachowicz-Stencel T, Synakiewicz A, Iżycka-Świeszewska E, Kobierska-Gulida G, Balcerska A. Malignant germ cell tumors associated with Swyer syndrome. Pediatr Blood Cancer. 2011;56:482-483. Epub 2010 Nov 15
41. Yada-Hashimoto N, Komura H, Nagata S, Kubo C, Fujita M, Kamiura S. Unexpected diagnosis of stage IIA dysgerminoma in streak gonad in a patient with Swyer syndrome: a case report. Gynecol Endocrinol. 2018;34:464-466. Epub 2017 Oct 31
42. Zhu J, Liu X, Jin H, Lu X. Swyer syndrome, 46,XY gonadal dysgenesis, a sex reversal disorder with dysgerminoma: a case report and literature review. Clin Exp Obstet Gynecol. 2011;38:414-418.
43. Zhu HL, Bao DM, Wang Y, Shen DH, Li Y, Cui H. Swyer's Syndrome with Mixed Ovarian Malignant Germ Cell Tumor and Ovarian Gonadoblastoma. Chin Med J (Engl). 2016;129:1752-1754.

CASE REPORT

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Novel *OBSL1* Variant in a Chinese Patient with 3M Syndrome: The c.458dupG Mutation May Be a Potential Hotspot Mutation in the Chinese Population

Yurong Piao[1], Rongmin Li[2], Yingjie Wang[2], Congli Chen[3], Yanmei Sang[3]

[1]National Center for Children's Health, Capital Medical University, Beijing Children's Hospital, Clinic of Immunity, Beijing, China
[2]Baoding Children's Hospital, Baoding Key Laboratory of Clinical Research on Children's Respiratory and Digestive Diseases Bao Ding, Clinic of Endocrinology, Hebei, China
[3]National Center for Children's Health, Capital Medical University, Beijing Children's Hospital, Department of Endocrinology, Beijing, China

What is already known on this topic?

3M syndrome is a rare autosomal recessive disorder. It is characterized by short stature, intrauterine growth retardation, low birth weight, dysmorphic facial features, and skeletal dysplasia. 3M syndrome includes three subtypes: type 1 with *CUL7* gene mutations, type 2 with *OBSL1* gene mutations, and type 3 with *CCDC8* gene mutations, accounting for approximately 77.5%, 16%, and 5%, respectively. There is no specific therapy for the disease. The effectiveness of growth hormone therapy for 3M syndrome is controversial.

What this study adds?

The c.427dupG mutation in the presented patient is a novel *OBSL1* variant. The c.458dupG mutation has been documented only in Chinese individuals, suggesting ethnic specificity. The phenotype and variant information of the five Chinese patients with c.458dupG mutation in the *OBSL1* gene are summarized. We suggest the c.458dupG mutation may be a hotspot mutation in the Chinese population.

Abstract

3M syndrome is an autosomal recessive disorder characterized by short stature and skeletal developmental abnormalities. A Chinese girl with 3M syndrome and a novel *OBSL1* (obscurin-like 1 gene) variant is presented. The patient is a 2-year-old girl who presented with short stature and had intrauterine growth retardation and low birth weight. Gene analysis revealed compound heterozygote mutations in the *OBSL1* gene: c.458dupG (p.L154Pfs*100) and c.427dupG (p.A143Gfs*111). The c.427dupG mutation is novel. The c.458dupG mutation has been documented in five cases, occurring only in Chinese individuals, suggesting ethnic specificity. In cases of children with short stature presenting with intrauterine growth retardation, low birth weight, and skeletal developmental abnormalities, 3M syndrome should be considered. The c.458dupG mutation may be a hotspot mutation in the Chinese population.

Keywords: Short stature, 3M syndrome, *OBSL1* gene, intrauterine growth retardation

Introduction

3M syndrome (MIM #273750, 612921, 614205) is a rare autosomal recessive disorder, which was first reported by Miller et al. (1). 3M syndrome is characterized by short stature, dysmorphic facial features, and skeletal dysplasia. 3M syndrome exhibits genetic heterogeneity. Based on different causative genes, it can be categorized into three subtypes: type 1 with *CUL7* gene mutations, type 2 with *OBSL1* gene mutations, and type 3 with *CCDC8* gene mutations, accounting for approximately 77.5%, 16%, and

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Address for Correspondence: Yanmei Sang MD, National Center for Children's Health, Beijing Children's Hospital, Capital Medical University, Department of Endocrinology, Beijing, China
E-mail: sangym_doc@126.com **ORCID:** orcid.org/0000-0002-6213-2661

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5%, respectively. However, about 1.5% of 3M cases have not yet been related to a definitive causative gene, suggesting a complex pathogenic mechanism (2).

The *OBSL1* gene consists of 22 exons with three splice variants designated as OBSL1A, B, and C. The encoded obscurin-like protein 1 (OBSL1), which is distributed in the cell membrane and encircling the nucleus, functions as a cytoskeletal adaptor protein that connects the cell interior to the cell membrane, contributing to the stability of the cellular cytoskeletal network. Approximately 200 cases of 3M syndrome have been reported to date, with only 24 cases in China. Among them, type 2 has been reported in over 50 patients worldwide, with only nine cases in China.

In this study, we report and analyze the clinical and molecular manifestations of a Chinese patient with 3M syndrome type 2 (OMIM 610991) caused by *OBSL1* gene mutations. We also review the relevant literature and summarize the other five Chinese patients with the same mutation in the *OBSL1* gene.

Case Report

The patient was a 2-year and 11-month-old girl of non-consanguineous parents. She was referred to our hospital due to her short stature. She was born at 38 weeks of gestation. Her birth weight, length, and head circumference were 2.0 kg [-3.1 standard deviation (SD)], 42 cm (-3.8 SD), and 34 cm. Prenatal examinations at five months of gestation indicated intrauterine growth retardation, with shorter-than-expected femur length for the gestational age. The mother had undergone an artificial abortion during her first pregnancy (G1P0) due to a diagnosis of short femur length.

At presentation, the patient's body weight was 9.6 kg (-3.6 SD), and her height was 79 cm (-3.8 SD). Serum basal insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 levels were 186 ng/mL (reference range: 51-303 ng/mL) and 4.86 µg/mL (reference range: 0.8-3.9 µg/mL). Growth hormone (GH) stimulation test was normal, with a peak GH level of 8.4 ng/mL. Radiographic examinations showed normal bone age, tubular bones, and vertebral bodies.

Recombinant human GH (rhGH) therapy was subsequently given over nearly five years. The patient's height increased by approximately 4 cm per year. Currently, when the patient was 7 years 6 months old, her weight was 19.0 kg (-1.7 SD), and her height was 108 cm (-3.2 SD), and she maintains normal intellectual development.

Genetic Analysis

Next-generation sequencing (NGS) was performed during the 7-year follow-up of the patient. Based on NGS analysis, two mutations, c.458dupG and c.427dupG, were detected in the *OBSL1* gene. The c. 458dupG variant is located in exon 1, resulting in a change in the p. L154Pfs*100 amino acid residue. This is a frameshift mutation that causes premature protein translation termination. According to the standards of the American College of Medical Genetics (ACMG) criteria, this mutation is considered pathogenic (PVS1 + PM3_Strong) and has been reported in clinical cases. The c. 427dupG variant is also located in exon 1, causing a change in the p. A143Gfs*111 amino acid residue. This is also a frameshift mutation that leads to premature protein translation termination. According to the ACMG standards, this mutation is also considered pathogenic (PVS1 + PM2 + PM3(Trans)). It is a newly identified mutation with a very low population frequency of 0.0000097. The patient carries compound heterozygous mutations in two pathogenic genes, resulting in premature protein translation termination, altered protein function, and associated clinical syndrome. Sanger validation of the variant gene is shown in Figure 1. Moreover, the amino acids in positions 154 and 143 are highly conserved among different species (Figure 2).

Discussion

We reported the clinical and genetic features of a Chinese girl with type 2 3M syndrome. The clinical manifestations of 3M syndrome lack specificity and predominantly involve short stature without accompanying intellectual impairment. In 2009, Hanson et al. (3) identified 10 cases with 3M syndrome who did not carry mutations in the *CUL7* gene. These ten individuals showed no discernible clinical distinctions compared to patients with *CUL7* gene mutations. Through high-density genome-wide SNP mapping, a second gene at 2q35-q36.1 was identified. This study reported seven mutations in *OBSL1* gene for the first time, including c.690insC (p. E231RfsX23), c.1149C→A (p.C383X), c.1273insA (p. T425NfsX40), c.1256_1265delGCACCGTGGC (p. R419PfsX10), c.1359insA (p. E454RfsX11), c.1463C→T (p.R489X), and c.2034_2035 delinsA (p.H679TfsX40). All these mutations were found within the first six exons of the gene (3).

3M syndrome patients manifest intrauterine growth retardation and short stature. In this study, the patient's primary presentation was short stature, along with a history of intrauterine growth retardation, low birth weight, and reduced birth length. In contrast, head circumference at birth remained within the normal range. These observations align with the established clinical characteristics of 3M syndrome. Most 3M syndrome patients demonstrate normal GH levels, and exhibit expected responses in GH stimulation

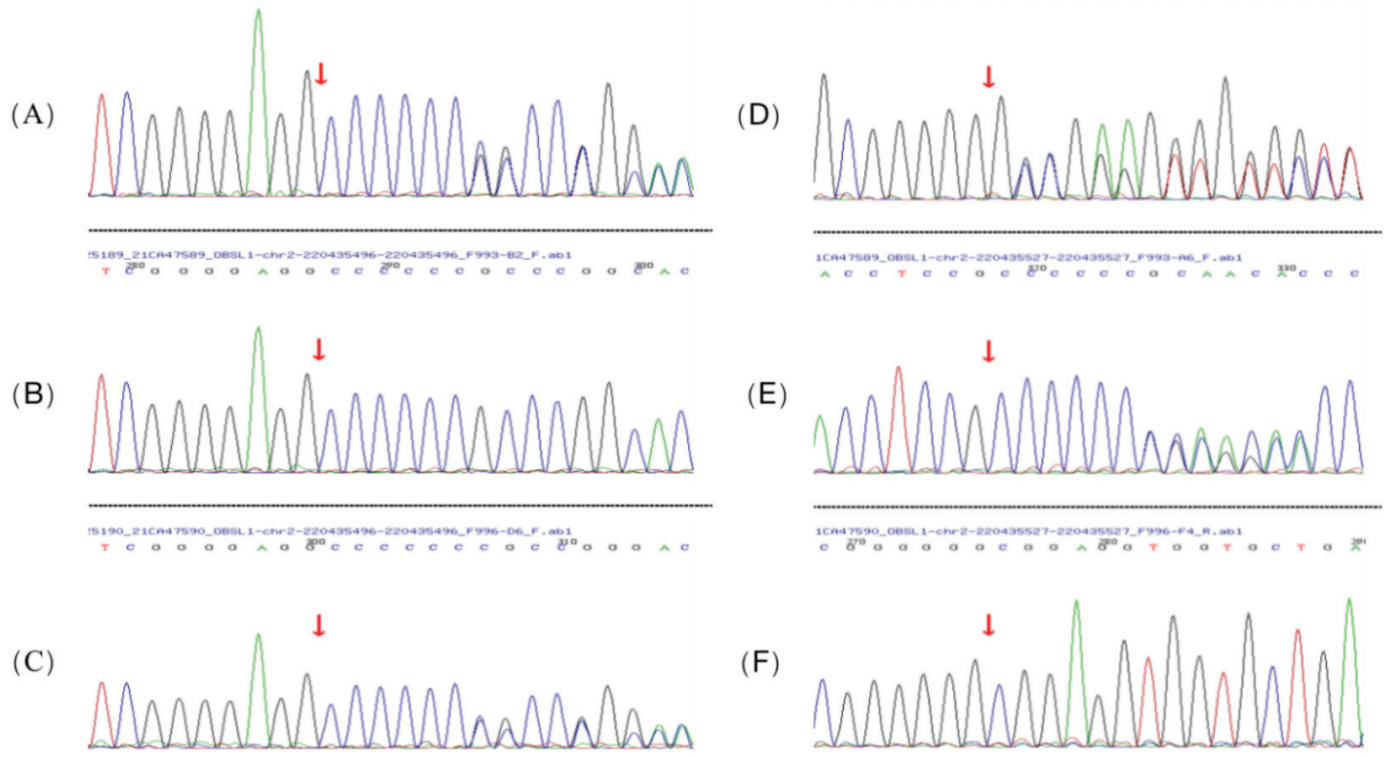

Figure 1. Sanger sequence showing the heterozygous mutations c.458dupG and c.427dupG. A) The patient bears the heterozygote c.458dupG frameshift mutation. B) The site is wild type in his father. C) The mother carries the heterozygote c.458dupG frameshift mutation. D) The child has the heterozygote c.427dupG frameshift mutation. E) The father carries the heterozygote c.427dupG frameshift mutation. F) The site is wild type in his mother

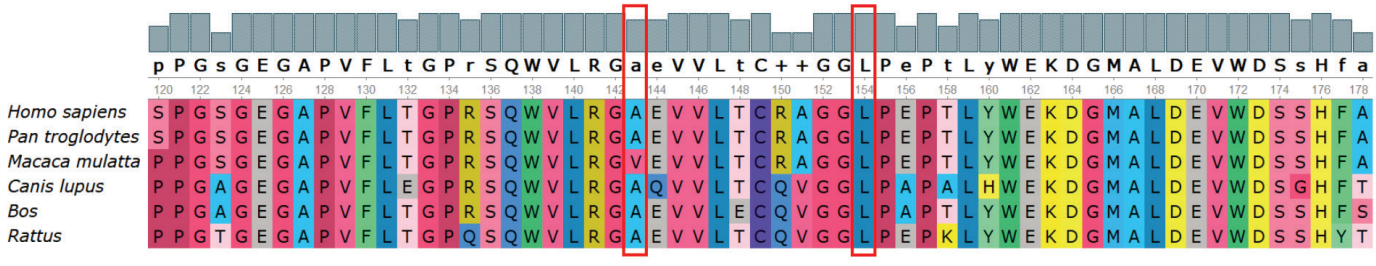

Figure 2. Conservation of the amino acid at positions 154 and 143 of the amino acid sequence among different species

tests. A minority have been reported to display inadequate stimulation test results (4,5). The peak GH response for the presented case was 8.4 ng/mL, and the IGF-1 level was average. According to the latest guidelines (6), this outcome rules out GH deficiency, indicating normal GH secretion.

All three subtypes of 3M syndrome may have a characteristic face, with minimal disparities among the subtypes. These features include a triangular face, pronounced forehead, flat nasal bridge, a round nasal tip, anteriorly tilted nostrils, elongated philtrum, thick lips, and prominent chin. Patients' dysmorphic facial features tend to be less noticeable when they grow up (7). In the current study, the patient exhibited a pronounced forehead, flat nasal bridge, and full round nasal tip at ten months of age (Figure 3A). By the age of five years, the pronounced forehead and depressed nasal bridge were less noticeable (Figure 3C). These findings emphasize the importance of clinicians keenly observing a child's facial appearance during their early years, which can aid in the prompt identification of this condition and subsequently facilitate timely genetic testing for a definitive diagnosis.

3M syndrome may exhibit skeletal developmental abnormalities, such as clinodactyly of the fifth finger, prominent heels, calf muscle protrusion, square shoulders, short neck, shortened chest cavity, reduced

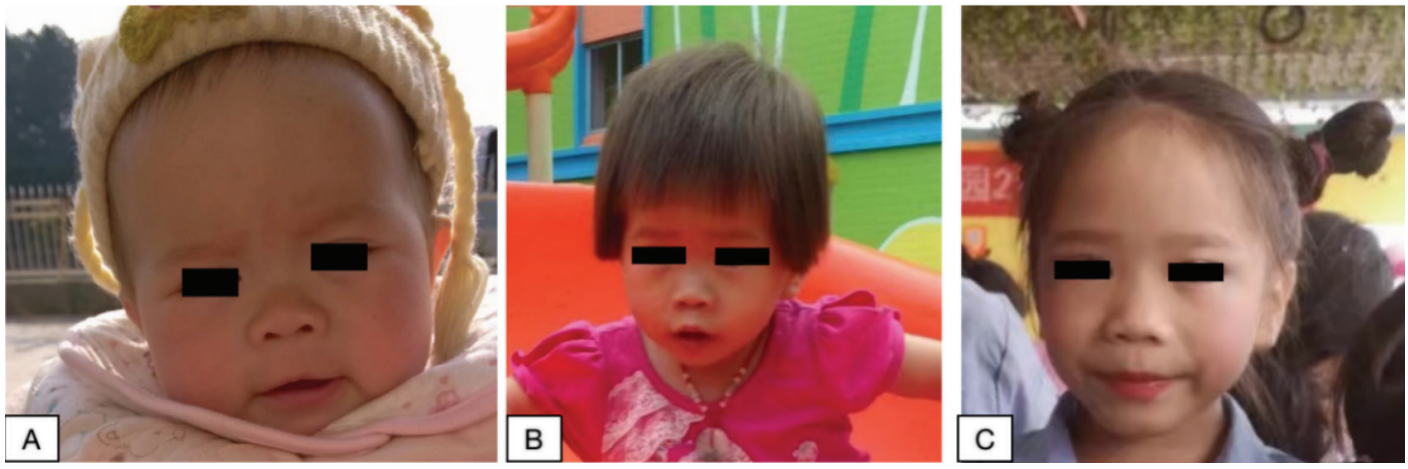

Figure 3. Facial appearance of our patient. A) 10 months old. A pronounced forehead, flat nasal bridge, and full round nasal tips were observed. B) Two years old. C) Five years old. A pronounced forehead and flat nasal bridge were less prominent

chest circumference, winged scapula, and anterior spinal protrusion. However, the patient in this study displayed milder clinical symptoms without any of these features.

In most 3M syndrome patients, skeletal X-ray assessments commonly reveal elongated tubular bones and tall vertebral bodies. Approximately 90% of patients display distinct characteristic alterations, including an elevated vertebral body height and a reduced anterior-posterior diameter. These alterations are especially noticeable in the lumbar vertebrae. Tüysüz et al. (8) analyzed 19 patients with 3M syndrome and found that tall vertebral bodies are more pronounced in children aged six years and older, as well as in adults. The patient in the present study did not manifest the characteristic vertebral changes, possibly due to her young age.

The *OBSL1* gene is located at 2q35 and consists of 22 exons with three splice variants designated as OBSL1A, B, and C. Its encoded product, obscurin-like protein 1 (OBSL1), comprises 1896 amino acid residues and is expressed in various cell types, including myocardium, skeletal muscle, brain tissue, and intervertebral discs. OBSL1 is distributed in the cell membrane and encircles the nucleus, functioning as a cytoskeletal adaptor protein that connects the cell interior to the cell membrane, contributing to the stability of the cellular cytoskeletal network.

More than 45 identified mutations in the *OBSL1* gene have been documented in the HGMD database. The reported mutation types include missense, nonsense, frameshift, deletion, and insertion mutations. Nine frameshift mutations have been reported. (c.35dupC, c.458dupG, c.690dupC, c.1039dupC, c.1125dupT, c.1260dupC, c.1273dupA, c.1359dupA, and c.2086_2088dupGGC). The presented case carries two mutations, c.458dupG (p. L154Pfs*100) and c.427dupG (p. A143Gfs*111). The two mutations are both frameshift mutations in exon 1, which lead to premature termination of protein translation. The c.458dupG frameshift mutation has been documented in only five cases to date (Table 1). All have occurred in Chinese individuals (9,10,11,12), which may be an indication of ethnic specificity and that this may be a hotspot mutation in the Chinese population.

Little is known about the mechanism underlying the short stature of 3M syndrome. The GH-IGF-1 axis in 3M syndrome appears normal, so the possibility of alternative pathways exists to induce abnormalities in growth plate chondrocyte development. Research into 3M syndrome pathogenesis has been multifaceted. In 2009, Huber et al. (13) revealed histological alterations in the growth plate of embryonic tibia in individuals with 3M syndrome. The researchers identified enlarged chondrocyte volume and increased density in both the resting and proliferative zones of the growth plate, accompanied by impaired extracellular matrix synthesis compared to normal cells. A separate investigation in 2013 proposed that the absence of autocrine IGF-2 functionality within the growth plates of children with 3M syndrome might contribute to their reduced stature (14). Subsequently, in 2014, Yan et al. (15) described the collaborative interaction among the proteins CUL7, OBSL1, and CCDC8, which form the 3M complex. OBSL1 acts as a bridging element between CUL7 and CCDC8. The function of the 3M complex is to uphold the integrity of microtubules - an essential aspect of mitosis and cytokinesis, crucial for normal cellular development. Notably, the study demonstrated that individual knockout of *CUL7*, *OBSL1*, and *CCDC8* genes didn't exacerbate conditions like mitotic delay, emphasizing their coordinated role within the same pathway. That may be why the three subtypes of

Table 1. Phenotype and variants information of five patients with c.458dupG mutation in *OBSL1* gene

Patient ID	Our case	1	2	3	4	5
Gender	Female	Female	Male	Female	Male	Female
Age	2 y 11 m	2 y	5 y 6 m	4 y	11 y 3 m	10 y 8 m
cDNA change	c.427dupG c.458dupG	c.458dupG (homo)	c.458dupG (homo)	c.1365-1387dup c.458dupG	c.1118G > A c.458dupG	c.690dupC c.458dupG
Birth length/cm	42	NA	45	NA	41	NA
Birth weight/g	2000	NA	2550	NA	2700	2300
Current height/cm	79 (-3.8 SD)	74 (-3.8 SD)	93 (-4.1 SD)	85 (-5.8 SD)	116.1 (-4.3 SD)	132.4 (-2.5 SD)
Current weight/kg	9.6	8.5	13.5	11.5	24	40.5
Growth retardation	+	+	+	NA	+	+
Triangular face	+	+	-	NA	+	+
Low nasal bridge	+	+	-	NA	+	+
Frontal bossing	+	+	-	NA	+	+
Normal intelligence	+	+	+	NA	-	+
Delayed bone age	-	-	+	NA	+	-
Bone change	-	-	-	NA	Smaller pelvic Long slender bones	Smaller pelvic Long slender bones

SD: standard deviation, NA: not applicable, y: year, m: month

3M syndrome exhibit closely similar clinical presentations. In a subsequent study by Wang et al. (16) in 2019, it was discovered that phosphorylated CCDC8 protein facilitated the formation of the 3M complex, prompting its relocation to the cell membrane. Once formed, the 3M complex initiated the ubiquitination-mediated degradation of LL5β. Disturbance in this process may give rise to altered microtubule dynamics, compromising cell migration and differentiation.

There is no specific therapy for the 3M syndrome. The effectiveness of GH therapy for 3M syndrome type 2 is controversial. Some researchers suggest that despite normal GH levels, some children with 3M syndrome exhibit inadequate GH stimulation, warranting GH treatment. Keskin et al. (5) reported a case of 3M syndrome type 2 treated with rhGH for six months at a dose of 7.5 IU/kg per week, resulting in a growth increment of 7 cm and a satisfactory growth rate. Clayton et al. (17) investigated the response to rhGH treatment in six individuals (including four patients who carried the causative mutation in the *OBSL1* gene). They found a small but significant increase in growth rate and height growth compared to the control group. However, some case reports indicate an ineffectiveness of GH treatment. Demir et al. (4) reported a child with homozygous *OBSL1* gene mutation (c.457_458delinsT) who underwent one year of GH treatment (dosage not mentioned), resulting in a mere 3 cm height increase. The presented patient received approximately five years of GH treatment, achieving a height increase of 4 cm per year with moderate effectiveness.

Due to the patient's average intelligence, the prenatal diagnosis of 3M syndrome remains debatable. For those with a family history or parents who are carriers of confirmed pathogenic genes and wish to have an unaffected child, a preimplantation genetic diagnosis could be considered, following ethical principles and informed consent. Regular prenatal ultrasound examinations are beneficial to early diagnosis. The growth rate of all long bones was observed to decrease. Two- and three-dimensional sonography can reveal shortened long bones and help detect mid-facial underdevelopment, aiding in prenatal diagnosis of 3M syndrome (18). Therefore, clinical prenatal examinations should focus on early identification and prompt genetic testing.

Conclusion

In summary, 3M syndrome is a rare disease primarily presenting with short stature. It should be considered when accompanied with intrauterine growth retardation, low birth weight, facial abnormalities in infancy, average head circumference, and skeletal developmental issues. Molecular analysis is needed to confirm the diagnosis. We found a novel mutation in the *OBSL1* gene in a Chinese patient. Moreover, the c.458dupG mutation in the *OBSL1* gene may be a hotspot mutation in the Chinese population.

Ethics

Informed Consent: Informed consent was obtained from the families of the study participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Yurong Piao, Rongmin Li, Yingjie Wang, Congli Chen, Yanmei Sang, Concept: Yanmei Sang, Design: Yanmei Sang, Data Collection or Processing: Yurong Piao, Rongmin Li, Yingjie Wang, Analysis or Interpretation: Yurong Piao, Congli Chen, Yanmei Sang, Literature Search: Yurong Piao, Congli Chen, Writing: Yurong Piao.

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References

1. Miller JD, McKusick VA, Malvaux P, Temtamy S, Salinas C. The 3-M syndrome: a heritable low birthweight dwarfism. Birth Defects Orig Artic Ser. 1975;11:39-47.
2. Huber C, Munnich A, Cormier-Daire V. The 3M syndrome. Best Pract Res Clin Endocrinol Metab. 2011;25:143-151.
3. Hanson D, Murray PG, Sud A, Temtamy SA, Aglan M, Superti-Furga A, Holder SE, Urquhart J, Hilton E, Manson FD, Scambler P, Black GC, Clayton PE. The primordial growth disorder 3-M syndrome connects ubiquitination to the cytoskeletal adaptor OBSL1. Am J Hum Genet. 2009;84:801-806. Epub 2009 May 28
4. Demir K, Altıncık A, Böber E. Severe short stature due to 3-M syndrome with a novel OBSL1 gene mutation. J Pediatr Endocrinol Metab. 2013;26:147-150.
5. Keskin M, Muratoğlu Şahin N, Kurnaz E, Bayramoğlu E, Savaş Erdeve Ş, Aycan Z, Çetinkaya S. A Rare Cause of Short Stature: 3M Syndrome in a Patient with Novel Mutation in OBSL1 Gene. J Clin Res Pediatr Endocrinol. 2017;9:91-94.
6. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, Cheung PT, Choong CSY, Cohen LE, Cohen P, Dauber A, Deal CL, Gong C, Hasegawa Y, Hoffman AR, Hofman PL, Horikawa R, Jorge AAL, Juul A, Kamenický P, Khadilkar V, Kopchick JJ, Kriström B, Lopes MLA, Luo X, Miller BS, Misra M, Netchine I, Radovick S, Ranke MB, Rogol AD, Rosenfeld RG, Saenger P, Wit JM, Woelfle J. Diagnosis, Genetics, and Therapy of Short Stature in Children: A Growth Hormone Research Society International Perspective. Horm Res Paediatr. 201;92:1-14. Epub 2019 Sep 12
7. Hasegawa K, Tanaka H, Higuchi Y, Yamashita M, Tsukahara H. Changes in facial appearance from neonate to adult in 3-M syndrome patient with novel CUL7 gene mutations. J Pediatr Endocrinol Metab. 2016;29:241-246.
8. Tüysüz B, Alp Ünkar Z, Turan H, Gezdirici A, Uludağ Alkaya D, Kasap B, Yeşil G, Vural M, Ercan O. Natural history of facial and skeletal features from neonatal period to adulthood in a 3M syndrome cohort with biallelic CUL7 or OBSL1 variants. Eur J Med Genet. 2021;64:104346. Epub 2021 Sep 28
9. Hu X, Li H, Gui B, Xu Y, Wang J, Li N, Su J, Zhang S, Song Y, Wang Y, Luo J, Fan X, Wang J, Chen S, Gong C, Shen Y. Prenatal and early diagnosis of Chinese 3-M syndrome patients with novel pathogenic variants. Clin Chim Acta. 2017;474:159-164. Epub 2017 Sep 29
10. Jiaorong Z, Shaohua C, Maozeng L, Xiaolei D, Xiaorong.Q. A case of 3-M syndrome treated with growth hormone therapy. Journal of New Medicine. 2021;52:293-295.
11. Yang LL, Liang SS. Study on pathogenic genes of dwarfism disease by next-generation sequencing. World J Clin Cases. 2021;9:1600-1609.
12. Xu N, Liu K, Yang Y, Li X, Zhong Y. Chinese patients with 3M syndrome: clinical manifestations and two novel pathogenic variants. Front Genet. 2023;14:1164936.
13. Huber C, Delezoide AL, Guimiot F, Baumann C, Malan V, Le Merrer M, Da Silva DB, Bonneau D, Chatelain P, Chu C, Clark R, Cox H, Edery P, Edouard T, Fano V, Gibson K, Gillessen-Kaesbach G, Giovannucci-Uzielli ML, Graul-Neumann LM, van Hagen JM, van Hest L, Horovitz D, Melki J, Partsch CJ, Plauchu H, Rajab A, Rossi M, Sillence D, Steichen-Gersdorf E, Stewart H, Unger S, Zenker M, Munnich A, Cormier-Daire V. A large-scale mutation search reveals genetic heterogeneity in 3M syndrome. Eur J Hum Genet. 2009;17:395-400. Epub 2008 Oct 29
14. Murray PG, Hanson D, Coulson T, Stevens A, Whatmore A, Poole RL, Mackay DJ, Black GC, Clayton PE. 3-M syndrome: a growth disorder associated with IGF2 silencing. Endocr Connect. 2013;2:225-235.
15. Yan J, Yan F, Li Z, Sinnott B, Cappell KM, Yu Y, Mo J, Duncan JA, Chen X, Cormier-Daire V, Whitehurst AW, Xiong Y. The 3M complex maintains microtubule and genome integrity. Mol Cell. 2014;54:791-804. Epub 2014 May 1
16. Wang P, Yan F, Li Z, Yu Y, Parnell SE, Xiong Y. Impaired plasma membrane localization of ubiquitin ligase complex underlies 3-M syndrome development. J Clin Invest. 2019;129:4393-4407.
17. Clayton PE, Hanson D, Magee L, Murray PG, Saunders E, Abu-Amero SN, Moore GE, Black GC. Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. Clin Endocrinol (Oxf). 2012;77:335-342.
18. Vimercati A, Chincoli A, de Gennaro AC, D'Addario V, Cicinelli E. 2D and 3D Ultrasonographic Evaluation of Fetal Midface Hypoplasia in Two Cases with 3-M Syndrome. Geburtshilfe Frauenheilkd. 2016;76:814-818.

CASE REPORT

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Clinical and Genetic Characteristics and Outcome in Patients with Neonatal Diabetes Mellitus from a Low Middle-income Country

Ishara Minuri Kumarasiri, Thabitha Jebaseeli Hoole, Manimel Wadu Akila Nimanthi, Imalka Jayasundara, Reha Balasubramaniam, Navoda Atapattu

Lady Ridgeway Hospital for Children Colombo, Clinic of Paediatric Endocrinology, Colombo, Sri Lanka

What is already known on this topic?

Neonatal diabetes mellitus (NDM) is a rare disorder where a genetic defect is identified in 80% of cases. The confirmation of these genetic defects plays a major role in further management and follow up of these patients.

What this study adds?

This study reports a genetic diagnosis in 96% of the cases of NDM, investigated retrospectively, in a cohort of patients from Sri Lanka.

Abstract

Neonatal diabetes mellitus (NDM) is a disorder characterized by persistent, severe hyperglycemia presenting during the first six months of life. These disorders are rare and the incidence is approximately 1 in 90,000 live births. The aim was to describe the clinical presentation, molecular genetics and outcome of patients with NDM from a single paediatric endocrine center from a low-middle income country, Sri Lanka. A retrospective study was conducted on patients diagnosed with NDM. Medical records were reviewed for demographic data and data on clinical, biochemical and genetic analysis. The majority (96%) who underwent mutation analysis had pathogenic genetic mutations on Sanger sequencing. Permanent NDM (PNDM) was diagnosed in 19 patients with three having a syndromic diagnosis. The most common mutation was in *KCNJ11*. The majority of patients with PNDM (63%) presented with severe diabetic ketoacidosis. All patients with Transient NDM remitted by six months of age. Nearly half (47%) with PNDM were switched to sulfonylurea therapy with good glycemic control (glycosylated haemoglobin A1c ranged 6-7.5%). Data from the Sri Lankan cohort is comparable with other populations. The majority of cases are due to *KCNJ11* mutations resulting in PNDM.

Keywords: Neonatal diabetes, genetics, clinical features, management, follow up

Introduction

Neonatal diabetes mellitus (NDM) is a disorder characterized by persistent, severe hyperglycemia presenting during the first six months of life (1,2,3). It can infrequently present between the ages of six months to one year (1,2,4). NDM is rare with a reported incidence of 1 in 90,000 live births (1,2,4). According to the phenotypic characteristic of insulin requirement the cases of NDM can be categorized as transient (TNDM) or permanent (PNDM) (3,5). In up to 80% of the cases, a genetic mutation has been recorded (2,3). These mutations cause neonatal diabetes mellitus through three major pathophysiological processes: malformed pancreas with abnormal beta cells; functional alteration of insulin secreting cells causing abnormal insulin synthesis; and destruction of beta cells (1,2).

Anomalies of the 6q24 locus and mutations of the *ABCC8* and *KCNJ11* genes are frequent genetic causes of abnormal beta cell function (1). These defects and *UDP 6* mutation

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Address for Correspondence: Navoda Atapattu MD, Lady Ridgeway Hospital for Children Colombo, Clinic of Paediatric Endocrinology, Colombo, Sri Lanka
E-mail: navodaa@gmail.com **ORCID:** orcid.org/0000-0002-3398-2536

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often cause TNDM (3,6). Mutations in *ABCC8* and *KCNJ11* are common in families without consanguinity (7). These mutations are also responsible for causing Maturity Onset Diabetes of the Young-MODY (7).

Genetic causes of PNDM remain obscure in most cases. In the non-consanguineous PNDM population, mutations in the ATP sensitive potassium channel and mutations in the *INS* gene are the most common finding, whereas in consanguineous families' mutations in the *INS*, *GCK* and *EIF2AK3* genes account for the majority of cases (3).

Studies on clinical and genetic characteristics of patients with NDM in South Asia are scarce. The diagnosis and genetic confirmation of these patients opens an avenue to a spectrum of management and follow up options (8). The aim of this study was to describe the clinical presentation, molecular genetics and long term follow up of a cohort of 24 patients with NDM from a single paediatric endocrine center in Sri Lanka.

Case Report

Study Design and Participants

Patients

An observational study of 24 registered cases of NDM presenting before one year of age were included.

Methods

Information collected from patients' records included demographic data (gender, age), clinical presentation, anthropometric measurements, laboratory findings at diagnosis, details on genetic analysis, treatment methods, comorbidities and adequacy of glycemic control on follow up. At diagnosis, the onset of diabetes mellitus and its complications were based on assessment of blood glucose levels, blood gases and electrolytes. Assessment of glycemic control was based on three monthly glycosylated haemoglobin A1c (HbA1c) levels. The values of HbA1c were categorized as good control (6-7.5%), fair control (7.51-9%), and poor control (>9%). Anthropometric measurements were taken by medical officers. Weight, height and body mass index were expressed as standard deviation scores according to the Centre for Disease Control and Prevention 2000 growth charts.

Genetic Analysis

Written, informed consent was taken from parents of patients who underwent genetic testing. Peripheral blood samples for genetic analysis were sent to University of Exeter Medical School, Exeter, United Kingdom. Genetic testing was undertaken for *ABCC8, KCNJ11, INS* and other mutations known to cause NDM. Analysis of the coding regions and exon/intron boundaries were done by targeted next generation sequencing. Sanger sequencing analysis and targeted next generation sequencing was used to confirm the genetic mutation in appropriate cases.

Patient no. 19 with trisomy 21 was found to have a recently recognised subtype of neonatal diabetes that is autoimmune but not human leukocyte antigen associated (Table 1). All other genetic causes were ruled out by sequencing and the NDM was found to be aetiologically caused by trisomy 21. Unfortunately, this patient was lost to follow up after eight months of age with the onset of the Covid pandemic. Therefore, this patient was not included in the study sample.

Genetic Analysis

Of the 24 patients with NDM, 19 (79%) were diagnosed with PNDM and 5 (21%) had TNDM. Of the five patients with TNDM, three showed a 6q24 mutation. The remaining two TNDM patients showed mutations, one in the *ABCC8* gene and the other patient in the *INS* genes. The genetic mutations responsible for the cases of PNDM are illustrated in Figure 1.

Twenty fathers of the 24 NDM patients underwent genetic analysis, of whom 12 were unaffected, five were heterozygous and two showed non paternity. One father was affected and he is currently on sulfonylurea therapy with well-controlled diabetes (HbA1c 6.8).

Clinical Presentation

Half of the 24 patients (50%) were female. and 12 (50%) were male. The age at presentation is shown in Figure

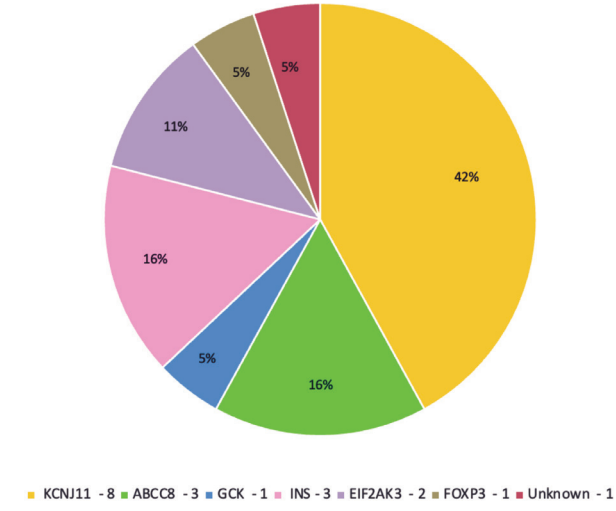

Figure 1. Genetic etiology of the patients with PNDM cohort
PNDM: permanent neonatal diabetes mellitus

2. A point of interest is that 4 out of the 5 patients with TNDM presented before four weeks of age. Furthermore, Patient 9 presented at 40 weeks and Patient 21 presented at 28 weeks. Both of these patients who presented after six months of age were positive for the *ABCC8* mutation.

Of the 19 patients with PNDM, 12 (63%) presented with severe diabetic ketoacidosis (DKA). Five of the 12 who presented with severe DKA were complicated with severe hypernatremia (serum sodium >170 mmol/L) and four patients suffered from stroke.

Complications and Comorbidities

Patient 7 who presented with severe DKA and severe hypernatremia underwent amputation of the toes of the right lower limb due to thrombosis of the peripheral vessels. The initial peripheral cyanosis extended above the ankle. However, with low molecular weight heparin infusion the dry gangrene was confined to the toes which required amputation.

Patient 13 with the *EIF2AK3* mutation causing Wolkott Rallison syndrome has had three episodes of liver failure, genu valgum, scoliosis and atlanto occipital subluxation requiring fixation. Despite this, he succumbed to severe pneumonia at eight years of age.

Patient 14, also with Wolkott Rallison syndrome, is currently being followed up at the clinic with a mean HbA1c of 8%. However, he is severely disabled with kyphoscoliosis and is wheel chair bound.

Patient 15 with immune-mediated polyendocrinopathy and enteropathy X-linked (IPEX) syndrome caused by a *FOXP3* mutation, presented with nephrotic syndrome and alopecia and was found to have NDM at 11 months of age. He passed away at 2 years of age due to severe pneumonia and pleural effusion complicated by sepsis.

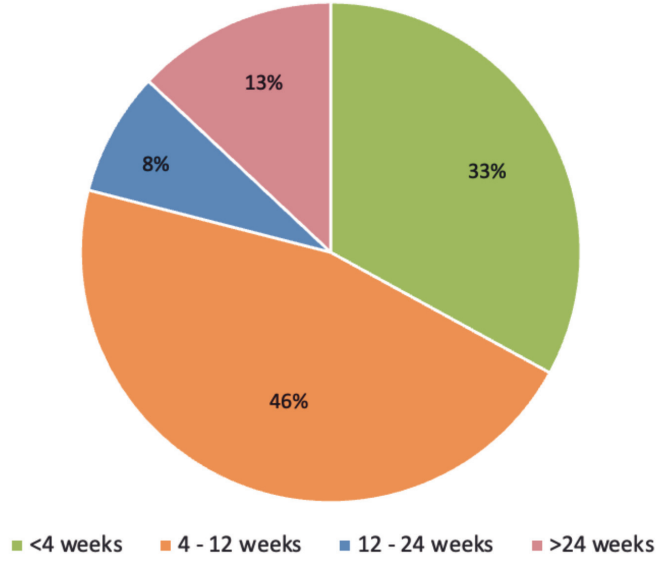

Figure 2. Age at presentation

Follow-up

All patients with TNDM were weaned off drugs by six months of age with regular monitoring of HbA1c levels.

Eight patients (42%) with PNDM, including Patient 20 whose genetic etiology is unknown, are receiving insulin with fair glycemic control (mean HbA1c of 8%). The nine patients who made a successful switch to sulfonylureas have good glycemic control with mean HbA1c of 7.3%. These patients are being regularly followed up at the clinic in view of evaluation for development of complications of diabetes and assessment of growth and development.

Of the four patients who suffered from a stroke at presentation, two have exhibited delay in achieving age-appropriate developmental milestones.

All patients, apart from Patient 14 with Wolkott Rallison syndrome, are showing satisfactory weight and height gain.

Discussion

In the past there have been a number of reports on clinical characteristics of NDM in European and Middle Eastern cohorts. To date there have been no published reports from Sri Lanka on comprehensive data on NDM, including genetic analysis. This is mainly because data on NDM, especially in view of long term follow up and genetic analysis is scarce. We present the clinical presentation, genetic analysis and glycemic control of patients with NDM from a single paediatric endocrine center in Sri Lanka.

Genetic Analysis

In comparison to the percentage of patients with PNDM in the Israeli cohort (57%), the American cohort (70%) and the Indian cohort (50%), our cohort had a frequency of PNDM of 83%, which is markedly higher than in the other cohorts (2,3,9). The most common genetic etiology for PNDM was mutations in the *KCNJ11* gene (42%). This finding agrees with previous records of European and Middle eastern populations where mutations of the *ABCC8, KCNJ11* and *INS* genes were found to be the most frequent etiologies for PNDM (3,7).

Of the 19 patients with PNDM, three tested positive for genetic mutations consistent with syndromic forms of PNDM. Two were positive for the *EIF2AK3* mutation and one was positive for the *FOXP3* mutation. Mutations in transcription factors involved in embryological development

Table 1. Results of genetic analysis and management of the neonatal diabetes mellitus cohort population

Patient no.	Status	Current management	Mutation	Maternal status	Paternal status
1	PNDM	Sulfonylurea therapy	Gene - *KCNJ11* Zygosity - Heterozygous HGVS description -NM_000525.3:c.602G > A p.(Arg201His) Location: GRCh37 (hg19) Chr11:g.17409037 Classification - Pathogenic	Unaffected	Affected
2	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* Location: Exon 1 DNA Description: c.149G > A Protein Description: p.Arg50Gln (p.R50Q) Consequence: Missense	Unaffected	Unaffected
3	PNDM	Insulin	Gene: *KCNJ11* Location: Exon 1 DNA Description: c.149G > C Protein Description: p.Arg50Pro (p.R50P) Consequence: Missense	Unaffected	Unaffected
4	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* DNA Description: c.2972G > A Protein Description: p.Ser991Asn (p.S991N) Consequence: Missense	Unaffected	Unaffected
5	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* Location: Exon 1 DNA Description: c.175G > A Protein Description: p.Val59Met (p.V59M) Consequence: Missense	Unaffected	Unaffected
6	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* DNA Description: c.136C > T Protein Description: p.(His46Tyr) Consequence: Missense	Unaffected	Unaffected
7	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* Location: Exon 1 DNA Description: c.136C > T Protein Description: p.(His46Tyr) Consequence: Missense	Unaffected	Unaffected
8	PNDM	Sulfonylurea therapy	Gene: *KCNJ11* Zygosity - heterozygous HGVS description - NM_000525.3:c.175G > A.p(Val59Met) Location - Chr11:g.17409464 Classification - pathogenic	Unaffected	Unaffected
9	PNDM	Sulfonylurea therapy	Gene - *ABCC8* DNA Description: c.265C > T Protein description: p.Arg89Cys (p.R89C) Consequence: Missense	Heterozygous	Single mother
10	PNDM	Insulin therapy	Gene: *ABCC8* Location: Exon 38 DNA Description: c.4568T > A Protein Description: p.(Val1523Glu) Consequence: Missense	Affected	Heterozygous
11	PNDM	Sulfonylurea therapy	Gene - *ABCC8* Zygosity - Heterozygous Inheritance - Maternal HGVS description - NM_001287174.1: c.970G > Ap.(Val324Met) Location -Chr11:g.17482076C > T Classification - Pathogenic	Heterozygous	Unaffected
12	PNDM	Insulin therapy	Gene: *GCK* Location: Exon 5 DNA Description: c.562G > A Protein Description: p.Ala188Thr (p.A188T) Consequence: Missense	Unaffected	Unaffected
13	PNDM	Deceased	Gene: *EIF2AK3* Location: Exon 13 DNA Description: c.2588T > G Protein Description: p.Leu863Ter (p.L863*) Consequence: Nonsense	Heterozygous	Non paternity
14	PNDM	Insulin therapy	Gene: *EIF2AK3* Location: Exon 14 DNA Description: c.2972G > A Protein Description: p.Ser991Asn (p.S991N) Consequence: Missense	Heterozygous	Heterozygous

Table 1. Continued

Patient no.	Status	Current management	Mutation	Maternal status	Paternal status
15	PNDM	Deceased	Gene: *FOXP3* Location: Exon 12 DNA Description: c.1236G > C Protein Description: p.Glu412Asp (p.E412D) Consequence: Missense	Heterozygous	Non paternity
16	PNDM	Insulin therapy	Gene: *INS* Location: Exon 3 DNA Description: c.265C > T Protein Description: p.Arg89Cys (p.R89C) Consequence: Missense	Unaffected	Unaffected
17	PNDM	Insulin therapy	Gene: *INS* Location: Exon 3 DNA Description: c.265C > T Protein Description: p.Arg89Cys (p.R89C) Consequence: Missense	Unaffected	Unaffected
18	PNDM	Insulin therapy	Gene - *INS* DNA Description: c.149G > A Protein Description: p.Arg50Gln (p.R50Q) Consequence: Missense	Heterozygous	Heterozygous
19	PNDM	Lost to follow up	Where all other genetic causes have been ruled out by sequencing, neonatal diabetes in patients with Down syndrome is aetiologically caused by trisomy 21. This recently recognised subtype of neonatal diabetes is autoimmune but is not HLA associated.	Not checked	Not checked
20	PNDM	Insulin therapy	No mutation identified	Not checked	Not checked
21	TNDM	Off drugs	Gene: *ABCC8* Location: Exon 8 DNA Description: c.1238C > G Protein Description: p.Thr413Ser (p.T413S) Consequence: Missense	Heterozygous	Unaffected
22	TNDM	Off drugs	Gene - *INS* Zygosity - Homozygous Inheritance - Biparental HGVS description - NM_001185098.1:c.-3 Location: GRCh37 (hg19)17A > C, p.? Chr11:g.2182518 Classification - uncertain significance	Heterozygous	Heterozygous
23	TNDM	Off drugs	Partial hypomethylation at the TND locus. This finding is consistent with a diagnosis of TND caused by a duplication of *6q24* of paternal origin.	Not checked	Not checked
24	TNDM	Off drugs	Methylation specific MLPA - Loss of methylation of the PLAGL1 DMR Dosage analysis - Normal dosage Informative markers tested - D6S1668 (6P25.1) - D6S1721 (6p24.1) - D65S1595 (6q15) - D6S280 (6q13) Maternal loss of heterozygosity for all Interpretation - MS-MLPA detected loss of methylation at the PLAGL1 DMR in the patients DNA sample. Microsatellite analysis showed no maternal contribution for 4 polymorphic chromosome 6 markers. The other 9 loci were not fully informative, but the patient was homozygous for all of them, consistent with uniparental disomy. This result confirms a diagnosis of TND, very likely due to paternal uniparental disomy at the *624* locus.	Not checked	Not checked
25	TNDM	Off drugs	Gene - *ZFP57* resulting in hypomethylation at the maternal 6q24 locus. Zygosity - Homozygous Inheritance - Biparental HGVS description -NM_001109809.2:c.844C > T p.(Gln282*) Location - Chr6:g.29641044 Classification - pathogenic	Heterozygous	Heterozygous

PNDM: permanent neonatal diabetes mellitus, TNDM: transient neonatal diabetes mellitus, TND: transient neonatal diabetes, MS-MLPA: Methylation-Specific Multiplex Ligation-dependent Probe Amplification, DMR: differentially methylated region

of the pancreas and elevated endoplasmic reticular stress giving rise to destruction of beta cells are two mechanisms involved in the pathogenesis of NDM in syndromic forms. Mutations in the *EIF2AK3* gene are responsible for beta cell destruction due to increased endoplasmic reticulum stress whereas mutations in the *FOXP3* gene are responsible for immune mediated damage to beta cells (2).

Sixty percent of the cases with TNDM tested positive for mutations in 6q24 gene, which is in keeping with data from Middle Eastern (70%) and American cohorts. Mutations in the *ABCC8* and *KCNJ11* genes were the second most common causes of TNDM in these cohorts (2,3). However, in our cohort, the other cases of TNDM were found to be due to a mutation in the *INS* and *ABCC8* genes.

Clinical Presentation

Cases of TNDM present earlier than PNDM (1,3,10). Moreover, patients with 6q24 mutation present earlier than those with potassium channel defects. This is evident in our cohort where 4 out of the 5 patients with TNDM presented before 4 weeks of age. However, the median age of presentation of cases with *KCNJ11* or *ABCC8* mutations was 9.6 weeks. However, presentation after six months of age has also been reported (2). Even in our cohort, two patients with *ABCC8* mutation and one patient with *KCNJ11* mutation presented after 6 months of age.

Presentation with DKA was more common in patients with PNDM when compared with cases of TNDM (10). Most (78.8%) of cases with mutations of *KCNJ11* or *ABCC8* presented with DKA whereas cases with TNDM due to overexpression of 6q24 did not present with DKA in the American cohort (2). This is evident in our cohort where none of the cases of TNDM presented with DKA. This is because the duration of insulinopenia is less in TNDM due to the earlier age of presentation (2) and the potassium channel mutations giving rise to PNDM cause a severe lack of insulin due to hyperpolarization of the membrane. This leads to marked reduction in insulin secretion whereas in TNDM there is only a reduction in beta cell function giving rise to a modest reduction in insulin secretion (3).

During the latter part of pregnancy insulin plays a major growth promoting role (6). Therefore, the lack of insulin leads to the low birth weight (1,3). All the patients in our cohort with K-ATP channel mutations had normal birthweight (birthweight > 2.5 kg).

Observation of growth parameters in our cohort revealed that all patients excluding Patient 14 with Wolkott Rallison syndrome showed satisfactory height and weight gain which may be attributed to proper glycemic control.

Complications and Comorbidities

Patients 1 and 2 with PNDM had a severe course complicated with DKA, hypernatremia and stroke. Fortunately, they are currently achieving age appropriate developmental milestones. They are on sulfonylureas therapy with good glycemic control (mean HbA1c 7%) (11).

Patients 3 and 12 also had a severe course complicated with severe DKA and stroke. However, their course was further convoluted with developmental delay and they are currently receiving multi-disciplinary care. They have a fairly controlled diabetes with a mean HbA1c of 8.5% (11).

The remaining 20 patients have no concerns regarding achievement of age-appropriate developmental milestones.

Follow-up

Successful treatment with sulfonylureas has been achieved in patients with *ABCC8* and *KCNJ11* mutations (1,2,3). The *KCNJ11* and *ABCC8* genes code for the Kir6.2 subunit and the SUR 1 ion-channel regulator subunit of the K-ATP channel respectively[4]. Sulphonylureas act on the K-ATP channel to induce closure of the channels, thus causing release of insulin from beta cells (6). Management with sulphonylureas has the advantages of reducing the incidence of hypoglycemia and improving the neurological and visual impairment in patients, if introduced early (1,12). The most frequently used sulphonyurea in NDM is glibenclamide (2). Nine out of the 12 patients with *ABCC8* or *KCNJ11* mutations in our cohort are currently on glibenclamide with a good glycemic control (mean HbA1c 7.3%) (11).

Eight patients with PNDM are currently on insulin administered according to the multiple daily dose regime. Initial management while the patient is on milk feeds is with long acting insulin agents. With the introduction of complimentary food, short acting insulin therapy prior to meals is initiated. It should be noted that due to financial and socio-economic constraints none of our patients are on insulin pumps. Furthermore, capillary sugars are checked using auto lancets as the luxury of continuous glucose monitors are not financially feasible in Sri Lanka. Glycemic control in this cohort is fair with mean HbA1c of 8%.

As this study was conducted in a single center in Sri Lanka the true incidence rate of NDM across the country cannot be estimated.

Conclusion

Data from our cohort is comparable with other populations. PNDM accounts for majority of the cases with mutations of

the *KCNJ11* responsible for a higher percentage of the cases. TNDM presents at an earlier age and remits by six months of age. The proportion of patients with PNDM presenting with severe DKA is higher than in patients with TNDM. Patients with *ABCC8* and *KCNJ11* mutation more frequently make a successful switch to sulfonylurea therapy.

A diagnosis of NDM should be considered in neonates and infants with persistent refractory hyperglycaemia. Genetic testing should be considered, as knowledge regarding the specific causative genetic mutation can appreciably modify the course of treatment. Close follow up is required in all patients with NDM in view of screening for complications and assessment of growth and development.

Ethics

Informed Consent: Written, informed consent was taken from parents of patients who underwent genetic testing.

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Footnotes

Authorship Contributions

Concept: Navoda Atapattu, Ishara Minuri Kumarasiri, Design: Navoda Atapattu, Ishara Minuri Kumarasiri, Thabitha Jebaseeli Hoole, Imalka Jayasundara, Data Collection or Processing: Navoda Atapattu, Ishara Minuri Kumarasiri, Reha Balasubramaniam, Manimel Wadu Akila Nimanthi, Analysis or Interpretation: Navoda Atapattu, Ishara Minuri Kumarasiri, Literature Search: Navoda Atapattu, Ishara Minuri Kumarasiri, Thabitha Jebaseeli Hoole, Imalka Jayasundara, Reha Balasubramaniam, Manimel Wadu Akila Nimanthi, Writing: Navoda Atapattu, Ishara Minuri Kumarasiri.

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References

1. Beltrand J, Busiah K, Vaivre-Douret L, Fauret AL, Berdugo M, Cavé H, Polak M. Neonatal Diabetes Mellitus. Front Pediatr. 2020;8:540718.
2. Dahl A, Kumar S. Recent Advances in Neonatal Diabetes. Diabetes Metab Syndr Obes. 2020;13:355-364.
3. Mouler M, Lebenthal Y, de Vries L, Yackobovitch-Gavan M, Averbuch NS, Fauret-Amsellem AL, Cavé H, Beltrand J, Polak M, Phillip M, Nimri R. Clinical characteristics, growth patterns, and long-term diabetes complications of 24 patients with neonatal diabetes mellitus: A single center experience. Pediatr Diabetes. 2022;23:45-54. Epub 2021 Dec 6
4. Lemelman MB, Letourneau L, Greeley SAW. Neonatal Diabetes Mellitus: An Update on Diagnosis and Management. Clin Perinatol. 2018;45:41-59. Epub 2017 Dec 16
5. Costa-Riquetto AD, de Santana LS, Franco PC, Jr ACS, Martio AE, Lisboa HRK, Kohara SK, Teles MG. Genetic and clinical features of neonatal and early onset diabetes mellitus in a tertiary center cohort in Brazil. Clin Genet. 2023;103:434-447. Epub 2022 Dec 23
6. Aguilar-Bryan L, Bryan J. Neonatal diabetes mellitus. Endocr Rev. 2008;29:265-291.
7. Warncke K, Eckert A, Kapellen T, Kummer S, Raile K, Dunstheimer D, Grulich-Henn J, Woelfle J, Wenzel S, Hofer SE, Dost A, Holl RW. Clinical presentation and long-term outcome of patients with KCNJ11/ABCC8 variants: Neonatal diabetes or MODY in the DPV registry from Germany and Austria. Pediatr Diabetes. 2022;23:999-1008. Epub 2022 Jul 23
8. Nyangabyaki-Twesigye C, Muhame MR, Bahendeka S. Permanent neonatal diabetes mellitus - a case report of a rare cause of diabetes mellitus in East Africa. Afr Health Sci. 2015;15:1339-1341.
9. Ganesh R, Suresh N, Vasanthi T, Ravikumar KG. Neonatal Diabetes: A Case Series. Indian Pediatr. 2017;54:33-36. Epub 2016 Nov 5
10. Polak M, Cavé H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis. 2007;2:12.
11. Kavthekar SO, Mali VT, Verma S, Kurane AB, Patil NB, Kulkarni SP. Evaluation of Microalbuminuria and Glycosylated Hemoglobin in the Assessment of Diabetes Control in Children with Type 1 Diabetes Mellitus Hospitalized with Diabetic Ketoacidosis. J Compr Ped. 2021;12:e109904.
12. Wu B, Xu W. Case report: Neonatal diabetes mellitus caused by KCNJ11 mutation presenting with intracranial hemorrhage. Front Neurol. 2013;14:1072078.

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Involvement of the Endocrine System is Common in Mitochondrial Disorders and Requires Long-term Comprehensive Investigations

Josef Finsterer

Neurology & Neurophysiology Center, Department of Neurology, Vienna, Austria

Keywords: mtDNA, mitochondrial disorder, endocrine organs, pituitary gland, diabetes

Dear Editor,

We read with interest the article by Papatya Çakır et al. (1) on a cross-sectional study of 26 patients with syndromic and non-syndromic mitochondrial disorders (MID). The syndromic MIDs included Leigh syndrome (n = 4), LHON (n = 2), MELAS (n = 2) and KSS (n = 1) (1). In 15 patients, MID was due to a mutation in the nDNA and in 10 patients to a mtDNA mutation (1). Of the 26 patients, 6 had endocrine involvement (1). These included ovarian insufficiency, central adrenal insufficiency, central hypothyroidism, diabetes mellitus and critical illness-related adrenal insufficiency (1). It was concluded that there is a high risk of developing hormonal deficiencies in MID (1). The study is excellent, but some points should be discussed.

The first point is that evaluation for endocrine disease in general, and specifically for endocrine involvement in MIDs, should include cerebral imaging, including the pituitary gland. Since central nervous system involvement is a common feature of MIDs and often manifests with morphologic or functional abnormalities in the hypothalamus or pituitary gland (empty sella, adenoma, or pituitary apoplexy) (2), cerebral imaging with special attention to these structures is essential.

The second point is that MID is usually a progressive disease with multisystem involvement that is either present at the onset of the disease or develops over the course of the disease. Therefore, endocrine involvement is not necessarily detectable in cross-sectional studies, but can only be found in long-term studies, which are preferable to cross-sectional studies. For this reason, it is recommended that MID patients be followed up regularly and prospectively screened for subclinical or mildly manifesting multisystemic disease, including endocrine involvement.

The third point is that MIDs often manifest with lactic acidosis, so we should know how many of the included patients had metabolic acidosis due to lactate overproduction in the muscle, cerebrum, myocardium or endocrine organs. With regard to lactic acidosis, we should know how many patients had elevated lactate not only in the serum but also in the cerebrospinal fluid (CSF). Elevated CSF lactate can also be documented by magnetic resonance spectroscopy, which usually shows a lactate peak and a reduced NAA peak (3). CSF lactic acidosis may secondarily affect pituitary functions.

The fourth point is that polycystic ovary syndrome (PCOS) may be an endocrine involvement in MIDs (4). How many of the included patients were diagnosed with PCOS?

The fifth point is that the patient with critical illness-related adrenal insufficiency should not be included in the group with endocrine involvement. If the adrenal insufficiency is due to critical illness neuropathy, it is not due to the underlying MID.

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Address for Correspondence: Josef Finsterer MD, Neurology & Neurophysiology Center, Department of Neurology, Vienna, Austria
E-mail: fifigs1@yahoo.de **ORCID:** orcid.org/0000-0003-2839-7305

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The sixth point is that no reference limits were given for most of the parameters analysed. Therefore, it is difficult to interpret whether a particular value is within or outside the normal range.

The seventh point is that patient 26 had a non-coding variant in MT-CR, suggesting that this nucleotide change was not pathogenic. How was the pathogenicity of the m.16519T > C variant confirmed?

In summary, this interesting study has limitations that put the results and their interpretation into perspective. Addressing these limitations could strengthen the conclusions and corroborate the message of the study. Endocrine system involvement is a common clinical manifestation of MID that can affect all endocrine organs and requires long-term follow-up, as it may not appear at the onset of the disease but may develop as the disease progresses. A comprehensive examination is required for early detection of endocrine disease in MID patients.

Footnotes

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References

1. Papatya Çakır ED, Ersıoy M, Çakır Biçer N, Gedikbaşı A. Endocrine Disorders in Children with Primary Mitochondrial Diseases: Single-Center Experience. J Clin Res Pediatr Endocrinol. 2024.
2. Ohkoshi N, Ishii A, Shiraiwa N, Shoji S, Yoshizawa K. Dysfunction of the hypothalamic-pituitary system in mitochondrial encephalomyopathies. J Med. 1998;29:13-29.
3. Maruyama S, Yamada T, Ishimoto Y, Hara H, Taniwaki T, Kira J. [A case of MELAS showing CSF pleocytosis associated with stroke-like episodes]. Rinsho Shinkeigaku. 1998;38:641-644.
4. Finsterer J. Mitochondrial Dysfunction in Polycystic Ovary Syndrome. Reprod Sci. 2023;30:1435-1442. Epub 2022 Oct 11

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In Response to: "Involvement of the Endocrine System is Common in Mitochondrial Disorders and Requires Long-term Comprehensive Investigations"

Esra Deniz Papatya Çakır[1], Melike Ersoy[1], Nihan Çakır Biçer[2], Asuman Gedikbaşı[3]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatric Nutrition and Metabolism, İstanbul, Turkey
[2]Acıbadem Mehmet Ali Aydınlar University Faculty of Health Sciences, Department of Nutrition and Dietetics, İstanbul, Turkey
[3]İstanbul University Institute of Child Health, Department of Pediatric Basic Sciences, Clinic of Medical Genetics, İstanbul University, İstanbul Faculty of Medicine, Department of Pediatric Genetics, İstanbul, Turkey

Keywords: Mitochondrial diseases, endocrine disorders, adrenal insufficiency, criticall illness

Dear Editor,

In response to Josef Finsterer's (1) letter, we would like to thank him for his interest in our study, and give a chance to us to emphasize and clarify a few points.

If we consider the first point in this letter regarding this article (2) we focused on all endocrinological problems in mitochondrial patients. We specifically mentioned pituitary imaging findings in patients with pituitary hormone deficiency. Two patients with central adrenal insufficiency and central hypothyroidism showed no abnormalities on their pituitary imaging. We provided detailed information about patients no. 20 and 21 under the subheadings of central adrenal insufficiency and central hypothyroidism in the results section of our article, stating that their pituitary magnetic resonance imaging (MRI) was normal.

Patient no. 20 had normal sella turcica contours and dimensions. Neurohypophysis showed normal hyperintensity. The infundibulum is in the midline, and its thickness were normal. We observe widespread T2-FLAIR pathological signal increases in both periventricular deep white matter and cortical deep white matter in brain MRI. This localization clearly identifies perivascular areas. Corpus callosum was thin. In the last control, there was no other hormone deficiency, especially pituitary hormones, and the annual growth rate was normal. Patient no. 21 has passed away. The brain MRI of the patient with global developmental delay revealed minimal hypoplasia in the brain stem and mild hypoplasia in the vermis. We observed variation in the cavum septum pellucidum et vergae. Both occipital localizations showed signal increases in FLAIR sequences in cortical-subcortical areas.

Secondly, our patient group includes different types of mitochondrial diseases, and their ages and follow-up periods were also variable. Our priority was to determine the current status in our group. This study is a preliminary study, and the follow-up of the patients is ongoing. Our studies targeting specific endocrine problems, including imaging, will continue in the future.

Lactic acidemia does not necessarily indicate the presence of mitochondrial diseases, a low value does not rule out mitochondrial disease, and a high value is a supportive finding (3). Lactate elevations in 5/26 patients were just

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Address for Correspondence: Esra Deniz Papatya Çakır MD, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatric Endocrinology, İstanbul, Turkey
E-mail: edpapatya@yahoo.com **ORCID:** orcid.org/0000-0003-4664-7435

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above the limit (21-82.56 mg/dL). Laboratory reference values were 4.5-20 mg/dL. Lactate levels were normal in 1/26 patients during follow-up, with a maximum of 10.3 mg/dL (patient no. 18). Although lactate levels in the remaining 20 patients decreased during treatment, basal lactate levels at diagnosis were 1.5 times higher than the upper limit (30.6-82.56 mg/dL). Among our two patients with pituitary hormone deficiency, patient no. 20 had a significant lactate elevation. Patient no. 21 also had a slightly elevated lactate level.

Twenty-two of our patients were in pubertal stage 1, and ten of them were female. These patients did not exhibit polycystic ovarian syndrome. There were two female patients in pubertal stage 5, one of whom had hypergonadotropic hypogonadism, and pubertal development and regular menstruation was achieved with pubertal hormone replacement therapy. To date, the other patient has no menstrual abnormalities and no clinical or biochemical hyperandrogenemia findings.

The hypothalamic-pituitary and adrenal axes play an important role in the stress response. Centrally activated hypercortisolism is considered the cornerstone of the human endocrine stress response. Adrenal insufficiency due to a critical illness does not develop in every patient, and although it is not a true hormone deficiency, we included it in this group because it developed in our patient with mitochondrial disease. Since critical illnesses and severe infections intensify the current energy crisis, leading to increased oxidative stress and decreased ATP synthesis, and obstruct the synthesis pathways, this situation is also regarded as evidence supporting mitochondrial insufficiency. In adrenal insufficiency due to a critical illness, the cortisol response, cortisol clearance, and cortisol receptor shift change within days. Although cortisol production is low, the cortisol value in circulation is high. We believe it is important to report this situation, as it indicates a functional cortisol deficiency. We conducted a retrospective study in our hospital's pediatric intensive care unit, examining data from 1,956 patients followed up in the tertiary intensive care unit for various reasons over a 5-year period, and found that only 79 patients developed critical illness-related adrenal insufficiency (4).

Laboratory reference values, primarily follicle-stimulating hormone, luteinizing hormone, estradiol, insulin-like growth factor-1, and IGFBP-3, vary according to age and gender. Therefore, they are given as standard deviations. For other parameters, average values that can be used for children aged 4-10 are added to the table below (Table 1).

Patient no. 26, who carries the 16519 T > C mutation in MT-CR, is diagnosed with mitochondrial disease and exhibits the Kearns-Sayre syndrome phenotype. The number of confirmed variants in MitoMap is only 96. Variants reported as disease-related but not yet confirmed form a large group of nearly a thousand variants. MitoMap classifies the variant

Table 1. Biochemical and hormonal profiles of the study population

	Number of patients (%)	Mean ± SDS or Median (min-max)
TSH (mIU/mL) (0.6-4.84)	26 (100)	2.49 ± 1.27
Free T4 (ng/dL)* (median, IQR) (0.97-1.67)	26 (100)	1.25 (0.85-4.09)
Free T3 (pg/mL) (2.53-5.22)	19 (73)	3.97 ± 0.95
ACTH (pg/mL)* (median, IQR) (7.2-63.3)	26 (100)	35 (4-365)
Cortisol (µg/dL)* (median, IQR) (6.2-22.6)	26 (100)	14.95 (5-68)
Calcium (mg/dL) (8.4-10.2)	26 (100)	9.79 ± 0.56
Phosphorus (mg/dL) (2.9-5.1)	26 (100)	4.57 ± 0.91
Magnesium (mg/dL) (1.7-2.2)	26 (100)	2.1 ± 0.18
ALP (U/L) (57-254)	26 (100)	203.5 ± 71.52
PTH (pg/mL) (15-65)	26 (100)	38.63 ± 23.59
25-hydroxy vitamin D (ng/mL)* (median, IQR) (20-80)	26 (100)	20 (4.71-94.2)
HbA1c %* (median, IQR) (4-6)	26 (100)	5.2 (4.7-7.25)
FSH (mIU/mL)* (median, IQR)	6 (23)	9.5 (3.05-280)
LH (mIU/mL)* (median, IQR)	7 (26.9)	8.3 (0.85-66)
IGF-1 (ng/mL) SDS* (median, IQR)	23 (88.5)	0.6 (-2.1-9.03)
IGFBP-3 (mg/L) SDS* (median, IQR)	22 (84.6)	-0.25 (-2.38-7.07)

*Non-parametric disturibition according to Kolmogorov-Smirnov test.
Normal values for laboratory parameters are indicated in parentheses beneath the parameter.
SDS: standard deviation score, TSH: thyroid-stimulating hormone, ACTH: adrenocorticotropic hormone, ALP: alkaline phosphatase, PTH: parathyroid hormone, IQR: interquartile range, FSH: follicle-stimulating hormone, LH: luteinizing hormone IGF-1: insulin-like growth factor-1, min-max: minimum-maximum

in question as disease-related and possibly pathogenic in silico, leading to its inclusion in the publication (5). However, it is important to remember that deletion-type mutations, another potential cause of the disease, are present in muscle tissue but not in peripheral blood.

We also agree with you that endocrine system involvement in mitochondrial diseases can affect all endocrine organs and may not occur at the beginning of the disease but may develop as the disease progresses, requiring long-term follow-up.

We thank you for your interest and suggestions in our study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Esra Deniz Papatya Çakır, Melike Ersoy, Concept: Esra Deniz Papatya Çakır, Melike Ersoy, Design: Esra Deniz Papatya Çakır, Melike Ersoy, Data Collection or Processing: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Asuman Gedikbaşı, Analysis or Interpretation: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Asuman Gedikbaşı, Literature Search: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Asuman Gedikbaşı, Writing: Esra Deniz Papatya Çakır.

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References

1. Finsterer J. Involvement of the Endocrine System is Common in Mitochondrial Disorders and Requires Long-term Comprehensive Investigations. J Clin Res Pediatr Endocrinol. 2024;16:514-515.
2. Papatya Çakır ED, Ersıoy M, Çakır Biçer N, Gedikbaşı A. Endocrine Disorders in Children with Primary Mitochondrial Diseases: Single-Center Experience. J Clin Res Pediatr Endocrinol. 2024.
3. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, Anselm I, Cohen BH, Falk MJ, Greene C, Gropman AL, Haas R, Hirano M, Morgan P, Sims K, Tarnopolsky M, Van Hove JL, Wolfe L, DiMauro S. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med. 2015;17:689-701.
4. Kiğılı G, Papatya Çakır ED, Akçay N, Sevketoğlu E. Retrospective evaluating of the patients with critical illnes-related corticosteroid insufficiency in pediatric intensive care unit. BMJ Paediatrics Open. 2024;8(Suppl 5):35-36.
5. MITOMAP: A Human Mitochondrial Genome Database. Last Accessed Date: 21.09.2024. Available from: http://www.mitomap.org,2023.https://www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsCodingControl

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