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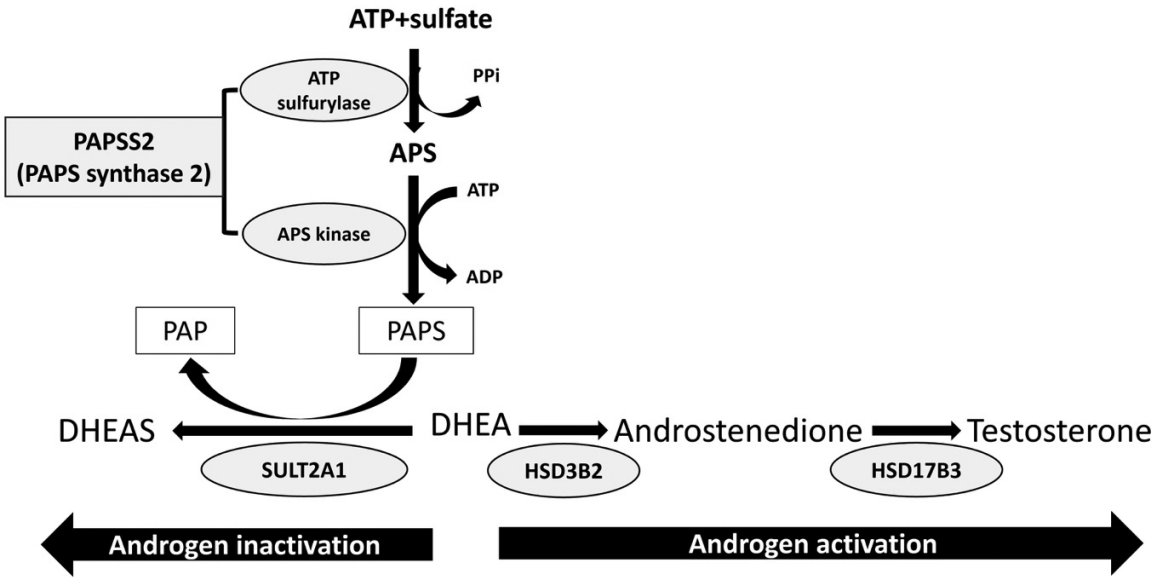

DHEAS production pathway. DHEA is the most abundant weak androgen precursor produced by the adrenal gland. Excess DHEA is sulfated by the enzyme SULT2A1, producing an inactive by-product, DHEAS, in the presence of a sulfate donor, PAPS. PAPS is generated by the PAPSS2 enzyme, which has ATP sulfurylase and APS kinase activities. This safeguarding reaction prevents excess DHEA from being used as a precursor to synthesize more potent androgens, such as androstenedione and testosterone, in adrenal, gonads and peripheral tissues

Bone Phenotype is Always Present But Androgen Excess is Less Frequently Seen in PAPSS2 Deficiency

Helvacıoğlu D and Güran T.

Page: 4-10

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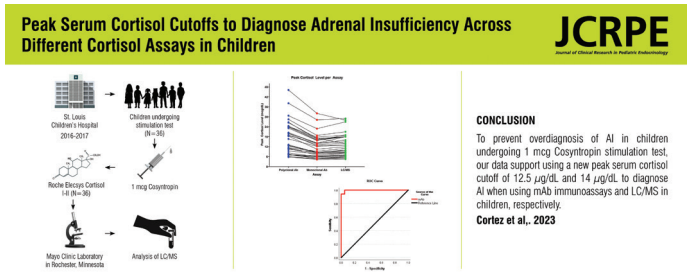

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CONTENTS

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CONTENTS

EDITORIAL

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J Clin Res Pediatr Endocrinol 2024;16(1):1-3

Diagnosis and Therapy in MCT8 Deficiency: Ongoing Challenges

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Keywords: Allan-Herndon-Dudley syndrome, MCT8 deficiency, triiodothyroacetic acid (Triac)

Dear Editor,

Monocarboxylate transporter 8 (MCT8) deficiency, or the Allan-Herndon-Dudley syndrome, is a rare neurodevelopmental and metabolic disorder caused by mutations in the *SLC16A2* gene located on the X-chromosome (1,2,3). MCT8 is a crucial thyroid hormone transporter for various tissues, including the brain. Hence, affected boys present with severe neurodevelopmental delay due to cerebral hypothyroidism (1,2,4). Early developmental milestones are often not reached and patients have severe intellectual and motor disability. The endocrine hallmark of MCT8 deficiency is increased serum (free) triiodothyronine (T3) concentrations, with reduced free thyroxine (T4) concentrations and normal to slightly elevated thyroid-stimulating hormone concentrations (4). The elevated (f) T3 concentrations lead to signs of thyrotoxicosis, such as tachycardia and being underweight (4). The disease has been associated with significant morbidity and mortality, with 30% of patients dying in childhood (4).

No approved therapeutic treatments are currently available for patients with MCT8 deficiency. T3-analogue Triac (3,3',5-tri-iodothyroacetic acid, or tiratricol) is not dependent on MCT8 for cellular influx and is, therefore, considered a good candidate for treatment (5,6). The Triac Trial I and subsequent real-world data showed that Triac safely normalizes serum T3 concentrations and ameliorates the symptoms of peripheral thyrotoxicosis in paediatric and adult male patients (7,8). Currently, the Triac Trial II (NCT02396459) is being conducted to explore potential beneficial effects in neurodevelopment in young patients (<30 months), the results of which are expected in 2024/2025. A small multicenter, double-blind, randomized, placebo-controlled trial [ReTRIACt trial (NCT05579327)], aiming to validate the effects of Triac on serum T3 concentrations, is currently recruiting.

In this issue of JCRPE, Ünsal and Hayran (9) present a new case-report of a patient with MCT8 deficiency. Their patient, diagnosed at the age of 14 months, exhibits typical characteristics of MCT8 deficiency, with a first presentation at 5 months of age including central hypotonia, developmental delay and feeding difficulties in combination with borderline normal thyroid function tests. This clinical presentation is in line with the median symptom onset at 4 months and median diagnostic delay of 14 months (10). This supports the claim of the authors that MCT8 deficiency is often overlooked and should be included in the differential diagnosis of young males with developmental delay and hypotonia. Since the "central" component of MCT8 deficiency is the most notable, most patients will be seen primarily by (pediatric) neurologists. Therefore, it is important to raise awareness of MCT8 deficiency in this profession. Moreover, there is large variation in the inclusion of the *SLC16A2* gene in multigene panels across different centers and countries (11), which implies that MCT8 deficiency is not always diagnosed after genetic testing. Consequently, *SLC16A2* should be included in all relevant genetic panels. Additionally, it should be explored whether modification of the newborn screening would allow for diagnosis of MCT8 deficiency immediately after birth (12,13).

In the current case-report, the patient was started on Triac with a maintenance dose of 133 mcg/kg/day (9). To assess neurodevelopmental outcomes, the Bayley Scales of Infant Developmental 3rd Edition (BSID-III) was used

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at baseline and various time points after treatment. No improvements in composite scores were observed after 12-months of treatment, although some improvements in a few developmental milestones were seen, such as improved head control. It is not possible to distinguish whether these changes would occur as part of the natural history or can be attributed to Triac treatment (4). Preclinical studies have indicated the potential of Triac in improving brain outcomes (5,14). Clinical studies sufficiently powered to detect clinically relevant neurodevelopmental changes, like Triac Trial II, might provide clues on potential benefits of early Triac treatment.

The authors report that after start of treatment with Triac, the FT3 values were variable and higher than expected (9), especially since Triac treatment aims to reduce FT3 values. The authors correctly assume that this is likely explained by cross-reactivity of Triac in the used FT3 immuno-assays. In 2022, Chan et al. (15) showed that all immuno-based T3 assays show significant interference with Triac, with assay-specific cross-reactivity profiles. Therefore, it is important to account for interference when interpreting FT3 results, both when unexpected high concentrations are measured as suggested by the authors, and also when T3 concentrations are measured in Triac treated patients. The relatively long time to achieve normal serum T3 values [7 months instead of the earlier reported 4 months by Groeneweg et al. (7)], should therefore also be cautiously interpreted since this was established by using only one immuno-based assay without applying any corrections for cross-reactivity. One way to account for interference is to employ a correction algorithm utilizing immuno-assays with different cross-reactivities towards Triac (7,8). Ultimately, the use of liquid chromatography mass spectrometry should be encouraged, as this allows to accurately distinguish Triac from T3 and prevents misinterpretation of thyroid function tests (15).

Overall, this case-report illustrates multiple challenges in the clinical management of patients with MCT8 deficiency (9). Despite the discovery of patients with mutations in MCT8 20 years ago, many unknowns remain, largely caused by the rarity of this disorder. Hence, like for all rare disorders, international collaboration to collect data in a uniform way on disease features and treatment outcomes is critical to advance diagnosis, management and therapeutic options for patients with MCT8 deficiency.

Acknowledgments

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Ethics

Authorship Contributions

Concept: Matthijs E.T. Freund, Floor van der Most, W. Edward Visser, Analysis or Interpretation: Matthijs E.T. Freund, Floor van der Most, W. Edward Visser, Literature Search: Matthijs E.T. Freund, Writing: Matthijs E.T. Freund, Floor van der Most, W. Edward Visser.

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References

1. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet 2004;364:1435-1437.
2. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004;74:168-175. Epub 2003 Dec 5
3. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, Ward J, Sanabria J, Marsa S, Lewis JA, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am J Hum Genet 2005;77:41-53. Epub 2005 May 11
4. Groeneweg S, van Geest FS, Abacı A, Alcantud A, Ambegaonkar GP, Armour CM, Bakhtiani P, Barca D, Bertini ES, van Beynum IM, Brunetti-Pierri N, Bugiani M, Cappa M, Cappuccio G, Castellotti B, Castiglioni C, Chatterjee K, de Coo IFM, Coutant R, Craiu D, Crock P, DeGoede C, Demir K, Dica A, Dimitri P, Dolcetta-Capuzzo A, Dremmen MHG, Dubey R, Enderli A, Fairchild J, Gallichan J, George B, Gevers EF, Hackenberg A, Halász Z, Heinrich B, Huynh T, Kłosowska A, van der Knaap MS, van der Knoop MM, Konrad D, Koolen DA, Krude H, Lawson-Yuen A, Lebl J, Linder-Lucht M, Lorea CF, Lourenço CM, Lunsing RJ, Lyons G, Malikova J, Mancilla EE, McGowan A, Mericq V, Lora FM, Moran C, Müller KE, Oliver-Petit I, Paone L, Paul PG, Polak M, Porta F, Poswar FO, Reinauer C, Rozenkova K, Menevse TS, Simm P, Simon A, Singh Y, Spada M, van der Spek J, Stals MAM, Stoupa A, Subramanian GM, Tonduti D, Turan S, den Uil CA, Vanderniet J, van der Walt A, Wémeau JL, Wierzba J, de Wit MY, Wolf NI, Wurm M, Zibordi F, Zung A, Zwaveling-Soonawala N, Visser WE. Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study. Lancet Diabetes Endocrinol 2020;8:594-605.
5. Kersseboom S, Horn S, Visser WE, Chen J, Friesema EC, Vaurs-Barrière C, Peeters RP, Heuer H, Visser TJ. In vitro and mouse studies supporting therapeutic utility of triiodothyroacetic acid in MCT8 deficiency. Mol Endocrinol 2014;28:1961-1970.
6. Groeneweg S, van Geest FS, Peeters RP, Heuer H, Visser WE. Thyroid Hormone Transporters. Endocr Rev 2020;41:bnz008.
7. Groeneweg S, Peeters RP, Moran C, Stoupa A, Auriol F, Tonduti D, Dica A, Paone L, Rozenkova K, Malikova J, van der Walt A, de Coo IFM, McGowan A, Lyons G, Aarsen FK, Barca D, van Beynum IM, van der Knoop MM, Jansen J, Manshande M, Lunsing RJ, Nowak S, den Uil CA, Zillikens MC, Visser FE, Vrijmoeth P, de Wit MCY, Wolf NI, Zandstra A, Ambegaonkar G, Singh Y, de Rijke YB, Medici M, Bertini ES, Depoorter

S, Lebl J, Cappa M, De Meirleir L, Krude H, Craiu D, Zibordi F, Oliver Petit I, Polak M, Chatterjee K, Visser TJ, Visser WE. Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: an international, single-arm, open-label, phase 2 trial. Lancet Diabetes Endocrinol 2019;7:695-706. Epub 2019 Jul 31

8. van Geest FS, Groeneweg S, van den Akker ELT, Bacos I, Barca D, van den Berg SAA, Bertini E, Brunner D, Brunetti-Pierri N, Cappa M, Cappuccio G, Chatterjee K, Chesover AD, Christian P, Coutant R, Craiu D, Crock P, Dewey C, Dica A, Dimitri P, Dubey R, Enderli A, Fairchild J, Gallichan J, Garibaldi LR, George B, Hackenberg A, Heinrich B, Huynh T, Kłosowska A, Lawson-Yuen A, Linder-Lucht M, Lyons G, Monti Lora F, Moran C, Müller KE, Paone L, Paul PG, Polak M, Porta F, Reinauer C, de Rijke YB, Seckold R, Menevşe TS, Simm P, Simon A, Spada M, Stoupa A, Szeifert L, Tonduti D, van Toor H, Turan S, Vanderniet J, de Waart M, van der Wal R, van der Walt A, van Wermeskerken AM, Wierzba J, Zibordi F, Zung A, Peeters RP, Visser WE. Long-Term Efficacy of T3 Analogue Triac in Children and Adults With MCT8 Deficiency: A Real-Life Retrospective Cohort Study. J Clin Endocrinol Metab 2022;107:1136-1147.
9. Ünsal Y, Hayran G. Impact of Early Intervention with Triiodothyroacetic Acid on Peripheral and Neurodevelopmental Findings in a Boy with MCT8 Deficiency. J Clin Res Pediatr Endocrinol 2024;16:116-122.
10. van Geest FS, Groeneweg S, Popa VM, Stals MAM, Visser WE. Parent Perspectives on Complex Needs in Patients With MCT8 Deficiency: An International, Prospective, Registry Study. J Clin Endocrinol Metab 2023;109:330-335.
11. Hoffman C, Berrens M. Landscape of genetic testing for monocarboxylate transporter 8 (MCT8) deficiency. 2023. Available from: https://www.egetis.com/wp-content/uploads/Hoffman_SSIEM_2023_Landscape-of-genetic-testing-for-MCT8-deficiency-004.pdf
12. Iwayama H, Kakita H, Iwasa M, Adachi S, Takano K, Kikuchi M, Fujisawa Y, Osaka H, Yamada Y, Okumura A, Hirani K, Weiss RE, Refetoff S. Measurement of Reverse Triiodothyronine Level and the Triiodothyronine to Reverse Triiodothyronine Ratio in Dried Blood Spot Samples at Birth May Facilitate Early Detection of Monocarboxylate Transporter 8 Deficiency. Thyroid 2021;31:1316-1321.
13. Yiu RS, Ling TK, Ko CH, Poon SW, Poon GW, Wong FC, Law CY, Iwayama H, Lam CW. Allan-Herndon-Dudley syndrome in Hong Kong: Implication for newborn screening. Clin Chim Acta 2023;551:117621.
14. Zada D, Tovin A, Lerer-Goldshtein T, Appelbaum L. Pharmacological treatment and BBB-targeted genetic therapy for MCT8-dependent hypomyelination in zebrafish. Dis Model Mech Dis Model Mech 2016;9:1339-1348.
15. Chan SL, Refetoff S, Babic N, Jin M, Garg U, Yeo KJ. Triiodothyroacetic Acid Cross-Reacts With Measurement of Triiodothyronine (T3) on Various Immunoassay Platforms. Am J Clin Pathol 2022;157:156-158.

REVIEW

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Bone Phenotype is Always Present But Androgen Excess is Less Frequently Seen in PAPSS2 Deficiency

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Abstract

3'-Phosphoadenosine 5'-phosphosulfate synthase 2 (PAPSS2) deficiency is a rare disorder due to biallelic pathogenic variants in the *PAPSS2* gene. This disorder was first described in 1998 by Ahmad et al. and Faiyaz ul Haque et al. To date, 79 patients with PAPSS2 deficiency have been reported. The main reported features of these patients are related to bone abnormalities and clinical/biochemical androgen excess. Disproportionate short stature and symptoms associated with spondylar skeletal dysplasia are the most common clinical features that require clinical attention. Androgen excess has been described much less commonly. This review summarizes the currently published clinical, molecular, and biochemical features of patients with PAPSS2 deficiency.

Keywords: PAPSS2, androgen excess, sulfation, brachyolmia, SEMD, DHEAS

Introduction

An 11.5-year-old Turkish girl was referred to our clinic because of short stature and low back pain. She was born at term with a birth weight of 2950 g [-0.9 standard deviation score (SDS)] after an uncomplicated pregnancy. Her parents were third cousins. At presentation her weight and height were 29.2 kg (-2.01 SDS) and 130.0 cm (-3.03 SDS), respectively. The ratio of upper-to-lower segment was 0.87 (-2.0 SDS). Systemic examinations were normal, except for limited dorsal bending. Her breast and pubic hair stages were Tanner IV. She had no acne or hirsutism. Her bone age was 12 years. Her predicted adult height was 141 cm, while the midparental height was 158 cm (-0.8 SDS). Initial tests for the etiology of her short stature, including routine biochemistry, full blood count, and thyroid function were all normal, and the karyotype was 46, XX. Insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 concentrations were normal. Due to her disproportionate short stature, a skeletal survey was also performed. Radiography of the lateral spine revealed findings suggestive for brachyolmia, including mild lumbar scoliosis, flattened vertebrae (platyspondyly) with rectangular vertebral bodies, irregular endplates and narrowed intervertebral disc spaces. Tubular bones were normal. Hormonal investigations using liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrated normal serum 17-hydroxyprogestrone (0.55 ng/mL; N: 0-1 ng/mL), androstenedione (1.07 ng/mL; N: 0.06-1.15 ng/mL), dehydroepiandrosterone (DHEA) (3.34 ng/mL; N: 0-3.4 ng/mL), total testosterone (0.24 ng/mL; N < 0.25 ng/mL), but low dehydroepiandrosterone sulphate (DHEAS) (77 ng/mL; N: 440-3320 ng/mL) concentrations. The plasma DHEAS/DHEA ratio was extremely low (22.6; N: 31-325). Sanger sequencing of the *PAPSS2* gene revealed a homozygous c.337dupG (A113Gfs*18) mutation. Menarche occurred at 12.1 years of age. She did not have clinical or biochemical evidence of androgen excess during two years of follow-up.

PAPSS2 Deficiency

Biosynthesis of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is essential for various biological processes, particularly in the regulation of sulfate metabolism and sulfation reactions in the body. These reactions are important in the production of substances, such as glycosaminoglycans, steroids and xenobiotics (1). PAPS is produced from ATP and

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inorganic sulfate by the action of PAPS synthetase (PAPSS). PAPSS has 2 domains; the ATP-sulfurylase domain catalyzes the formation of the 5'-adenosine phosphosulfate (APS) intermediate from ATP and inorganic sulfate. The APS kinase domain then further phosphorylates APS by an additional ATP molecule to generate PAPS (Figure 1). This is the rate-limiting step in PAPS biosynthesis. Two different PAPSS isoforms have been identified in human tissues, known as PAPSS1 and PAPSS2 (1). PAPSS1 is the predominant isoform and is found in various human adult tissues. In contrast, PAPSS2 exhibits a more restricted expression pattern and appears to be the major variant in growth plate cartilage (2). The catalytic efficiency of PAPSS2 is 10 to 15 times as great as that of PAPSS1 (2). PAPSS1 deficiency has so far not been shown to be directly linked to the etiology of any human disease. However, the physical interaction between the two enzymes has been shown to influence the cellular localization, and possibly the function, of PAPSS2 (3).

PAPSS2 deficiency was first described by Ahmad et al. (4) in 1998 in a large consanguineous family. The main characteristics of the 16 affected family members (11 males and 5 females) include spondyloepiphyseal metaphyseal dysplasia (SEMD), severe short stature and early-onset joint problems. The authors classified this distinctive autosomal recessive form of SEMD as SEMD Pakistani type. In the same year, Faiyaz ul Haque et al. (5) showed that the molecular defect in this family was a homozygous c.1439C > A; p.Ser480* mutation in the *PAPSS2* gene (Table 1). Ten years later, the second paper on genetically proven PAPSS2 deficiency was published by Noordam et al. (6). They reported a Turkish girl who presented with premature pubarche (early development of pubic hair), advanced bone age, and disproportionate short stature due to skeletal dysplasia affecting the vertebrae with no epiphyseal or metaphyseal changes. The bone phenotype of that patient was more compatible with brachyolmia (*"Brachy" in Greek means "short", and "olmós" means "spine";* osteochondrodysplasia characterized by generalized platyspondyly without significant long bone abnormalities). During follow-up, she developed hirsutism, acne and secondary amenorrhea, meeting the diagnostic criteria for polycystic ovary syndrome (PCOS). In this patient, LC-MS/MS detected high concentrations of androstenedione and testosterone, upper limit of normal DHEA and very low DHEAS. In terms of steroidogenesis, they showed that the etiology of clinical and biochemical androgen excess involved impaired DHEA sulfation, which results in more DHEA available for 3β-hydroxysteroid dehydrogenase activity to produce androstenedione, which in turn is converted to more potent androgens, such as testosterone and dihydrotestosterone by the action of 17β-hydroxysteroid dehydrogenase and 5α-reductase enzymes, respectively (Figure 1) (6).

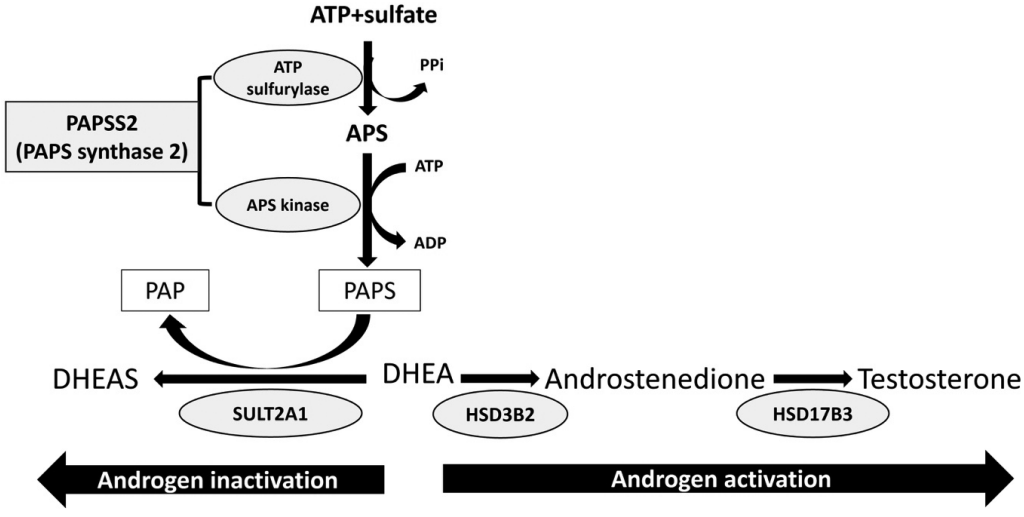

Figure 1. DHEAS production pathway. DHEA is the most abundant weak androgen precursor produced by the adrenal gland. Excess DHEA is sulfated by the enzyme SULT2A1, producing an inactive by-product, DHEAS, in the presence of a sulfate donor, PAPS. PAPS is generated by the PAPSS2 enzyme, which has ATP sulfurylase and APS kinase activities. This safeguarding reaction prevents excess DHEA from being used as a precursor to synthesize more potent androgens, such as androstenedione and testosterone, in adrenal, gonads and peripheral tissues

DHEA: dehydroepiandrosterone, DHEAS: dehydroepiandrosterone sulphate, APS: adenosine 5-phosphosulfate, PAPS: 3-phosphoadenosine 5-phosphosulfate, PAP: 3-phosphoadenosine 5-phosphate, PPi: pyrophosphate, PAPSS2: 3-phosphoadenosine 5-phosphosulfate synthase 2, SULT2A1: DHEA sulfotransferase

The molecular diagnosis of new patients with PAPSS2 deficiency has increased understanding of both the bone and steroid hormone characteristics of this disease. In Table 1, molecular and basic clinical features of patients with genetically confirmed PAPSS2 deficiency are presented in chronological publication order. To date, 79 (41 males, 38 females) patients with PAPSS2 deficiency have been reported, of whom 29 (36.7%) were Turkish patients. In the reported patients, 32 pathogenic *PAPSS2* mutations were identified, 13 (40%) of which led to frameshift and 5 (15.6%) to the formation of truncated proteins. Although the functional consequences of *PAPSS2* variants were rarely studied by *in vitro* assays, more than half of the pathogenic variants (59.3%) detected in the *PAPSS2* gene cause severe, complex alterations in the gene and cause disease. The mutations that diminish the ATP-sulfurylase domain and APS kinase domain are mainly related to 389-611th and 41-196th aminoacid residues of PAPSS2, respectively. The mutations

Table 1. Clinical, molecular and biochemical characteristics of previously reported patients with *PAPSS2* mutations

***PAPSS2* variant (NM_004670.3)**	**Gender**	**BO/SEMD phenotype**	**Androgen excess**	**Ref.**
S480*	11 M, 5 F (Pakistani)	+	No data on hormones	(4)
T48R/R329*	F (Turkish)	+	PP, high androstenedione and testosterone, low DHEAS, upper normal DHEA	(6)
A113Gfs*18, IVS3+2delT, V206Sfs*9/R437Gfs*19, K161Rfs*6/I221Sfs*40	2 M, 4 F (3 Turkish, 2 Japanese and 1 Korean)	+	(-) Clinical androgen excess, low DHEAS	(14)
R329*	3 M, 2 F (Turkish)	+	1 F patient had clinical androgen excess and high testosterone, all had low DHEAS, but normal DHEA	(15)
L76Q, R129Lfs*25,V364Rfs*18, A113Gfs*18, V540N, E183K, Q211Cfs*11, C43Y, c.27+3A>C, F125Sfs*24	5 M, 8 F (12 Turkish, 1 Lebanese)	+	Evaluated in 6/13 pts. 2 patients had hypertrichosis and acnea, 1 had PP. No hormonal w/o	(16)
W462Cfs*3/G270D	2 M (European?)	+	(-) Clinical androgen excess, normal androgens, normal DHEA and testosterone, low DHEAS, p.Trp462Cysfs* heterozygous mother have clinical androgen excess	(10)
H355Ifs*5	M (Kurdish)	+	(-) Clinical androgen excess, high DHEA, no DHEAS data	(9)
R334*, c.639+1G>T	3 M, 4 F (Turkish)	+	All had low DHEAS, 2 had clinical androgen excess, no DHEA data available	(17)
L440Wfs*12	F (Turkish)	+	(+) Clinical androgen excess (PP), high DHEA, low DHEAS, normal testosterone and androstenedione	(18)
G270D, R41*, F283V, G538Afs*4, A493V, R334*, W462Cfs*3, R129P	10 M, 8 F (6 Norwegian, 5 British, 1 Pakistani, 1 Algerian, 1 French, 1 African, 1 Egyptian, 2 Eastern European)	+	Evaluated in 10/18 pts. 2 had clinical androgen excess who had G270D mutation, all patients had low DHEAS, No DHEA measured, 3 had high testosterone or androstenedione	(19)
R238*	F (Chinese)	+	ND	(20)
H496P	1 M, 1 F (Jordanian)	+	No clinical or biochemical androgen excess, high DHEA and low DHEAS	(21)
R346P	3 M, 3 F (Pakistani)	+	ND	(22)

DHEA: dehydroepiandrosterone, DHEAS: dehydroepiandrosterone sulphate, PP: premature pubarche, ND: not defined, M: male, F: female, BO: brachyolmia, SEMD: spondyloepimetaphyseal dysplasia

identified so far appear to be distributed throughout the gene without hotspot regions. There are some founder mutations in Turkish patients (A113Gfs*18, R129Lfs*25, R334*, W462Cfs*3, R238*). High consanguinity and founder mutations may explain the predominance of Turkish patients in the published literature.

The next section of this article will focus on the two main clinical presentations of PAPSS2 deficiency based on published data from patients: skeletal manifestations and steroid metabolome alterations.

Skeletal Manifestations of PAPSS2 Deficiency

Skeletal manifestations are present with variable severity in all patients with PAPSS2 deficiency. The main cause of cartilage and bone disease in PAPSS2 deficiency is the impaired biosynthesis and deposition of sulfated proteoglycans in the cartilage and extracellular bone matrix (7).

Formerly, PAPSS2-related skeletal dysplasia was associated with SEMD Pakistani type, which includes the former "recessive brachyolmia", as well as the older entities "Toledo type" and "Hobaek type" brachyolmia. In the 2023 revision of the Nosology of Genetic Skeletal Disorders, it is now classified as part of the group of sulfation disorders (NOS 04-0050; OMIM 612847) (8).

The most important clinical feature is disproportionately short stature with a short spine associated with variable symptoms, including pain, stiffness and spinal deformity, such as kyphoscoliosis. Short trunk dwarfism becomes apparent in childhood, usually after the age of two years (8). Patients may have a short femur prenatally, but a short spine phenotype develops later in childhood. Genu varum, genu valgum, enlargement in the knee and ankle joints, patella dislocations, limited flexion in the metacarpophalangeal joints and early-onset osteoarthropathic changes in the hand and knee have been described. There are four different bone phenotypes associated with PAPSS2 deficiency:

1. SEMD where both vertebrae and long bones are affected,

2. Symptomatic brachyolmia with dysplastic changes limited to the spine involving minimal epimetaphyseal changes visible only on X-ray,

3. Symptomatic brachyolmia with dysplastic changes limited to the spine,

4. Subclinical brachyolmia with radiologic changes only.

Skeletal surveys may show severe platyspondyly with elongated vertebral bodies, irregular endplates, narrow intervertebral disk spaces, overfacing of the pedicles, and lumbar spinal canal stenosis. Spondylar dysplastic changes may progress with age. The bone age maybe advanced. Short femoral neck, bowed tibia, short and broad ilia, dumbbell deformity of the long and short tubular bones, and irregularities of epiphyseal ossification have been described. Metaphyseal longitudinal striations in the proximal femora are distinctive. Other long bones may show mild epiphyseal flattening and metaphyseal irregularities (9).

Although spondylar dysplasia is a "*sine qua non*" characteristic of the disease, the severity of skeletal manifestations does not correlate with the severity of the *PAPSS2* mutation detected. Thus, there is no clear genotype-phenotype relationship in terms of bone findings. As seen in the patient presented above, the homozygous frameshift mutation led to a brachyolmia phenotype without epiphyseal-metaphyseal changes. *PAPSS2* mutations detected in patients with brachyolmia or SEMD phenotype are shown in Table 2.

Steroid Metabolome Alterations in PAPSS2 Deficiency

Low plasma DHEAS is the most consistent biochemical feature of patients with PAPSS2 deficiency published to date. Low DHEAS concentrations are not associated with the type of the mutation in *PAPSS2*. The DHEAS/DHEA ratio

Table 2. Skeletal phenotypes in patients with *PAPSS2* mutations

***PAPSS2* mutations related to brachyolmia**	***PAPSS2* mutations related to SEMD**
T48R/R239X (6)	S480*/S480* (4)
K161Rfs*6/K161Rfs*6 (14)	R239X/R239X (15)
A113Gfs*18/A113Gfs*18 (14)	G270D/W462fs*3 (10)
V206Sfs*9/R437Gfs*19 (14)	G270D/G270D (19)
c.381 + 2delT/c.381 + 2delT (14)	R239*/R239* (20)
V540D/V540D (16)	
C43Y/C43Y (16)	
V364Rfs*18/V364Rfs*18 (16)	
R129Lfs*25/R129Lfs*25 (16)	
Q211Cfs*11/Q211Cfs*11 (16)	
c.27 + 3A > C/c.27 + 3A > C (16)	
F125Sfs*24/F125Sfs*24 (16)	
H355Ifs*5/H355Ifs*5 (9)	
G270D/R129P (19)	
G270D/G270D (19)	
G270D/W462Cfs*3 (19)	
R41X/R41X (19)	
R334X/R334X (19)	
F283V/F283V (19)	
L440Wfs*12/L440Wfs*12 (18)	
R346P /R346P (22)	
H496P/H496P (21)	

is significantly lower than in the healthy population, but this ratio varies depending on the plasma concentration of DHEA. DHEA can be normal or elevated. In 7 of 17 PAPSS2 deficiency patients with published DHEAS measurement data, DHEAS concentrations were below the measurable limit. In the other 10 patients, the median (range) DHEAS concentration was 10.5 (3.9-48.7) ug/dL. In seven patients with simultaneous DHEA and DHEAS measurements, the median DHEAS/DHEA ratio was 10.5 (4.4-50.6). Concentrations of other adrenal or gonadal androgens, including androstenedione and testosterone, are also variable, and this variability is not associated with the type and location of the *PAPSS2* mutation. Elevated testosterone, androstenedione or DHEA concentrations were reported in nine patients (11.3%). An important point to note is that in some reports the androgen profile of patients was not analyzed in detail (Table 1), but the majority of patients with hormone profile data had normal androgen values, as seen in the case reported at the beginning of this review. Oostdijk et al. (10) reported two brothers with PAPSS2 deficiency who had normal basal androgen concentrations but had higher androgen concentrations after DHEA stimulation testing compared to healthy controls. However, besides normal basal androgens, these patients also had no clinical symptoms of androgen excess.

Clinical signs of androgen excess (acnea, hypertrichosis, premature pubarche, PCOS, and menstrual irregularity) have been reported in 10 (12.6%) of the 79 patients reported so far (Table 1). Premature pubarche was described in three patients (3.7%). As seen in the patient presented at the beginning of this review, the majority of the patients with genetically confirmed PAPSS2 deficiency do not show clinical or biochemical signs of androgen excess.

The prevalence of hirsutism and PCOS in the general population is approximately 5-25% and 6-20% respectively. Premature pubarche is also not uncommon, with a prevalence of 4-7%, and has been reported as 4.3% in Turkey (11,12). Therefore, based on the data of the currently described patients, it seems difficult to speak of an increased frequency of androgen excess in patients with PAPSS2 deficiency compared to the general population. Nevertheless, given that sulfate conjugation is an important mechanism that prevents potent androgen synthesis by inactivating the precursors that can be used in the canonical or backdoor pathway of androgen synthesis, including DHEA, pregnenolone, 17-OH-Pregnenolone, androsterone, and androstenediol, clinical or biochemical androgen excess findings should be suspected and investigated in patients with PAPSS2 deficiency (Figure 2) (13). It is not clear why clinical/biochemical signs of androgen excess are not seen in all patients with PAPSS2 deficiency, although theoretically the pool of androgen precursors that are not sulfated and therefore available for active androgen biosynthesis is larger. One possibility is that, although the catalytic activity of PAPSS1 is 10-15-fold lower than that of PAPSS2, in the absence of PAPSS2 activity, the ubiquitously expressed PAPSS1 can compensate for the missing enzyme and supply tissues with sufficient PAPS. However, this rescue

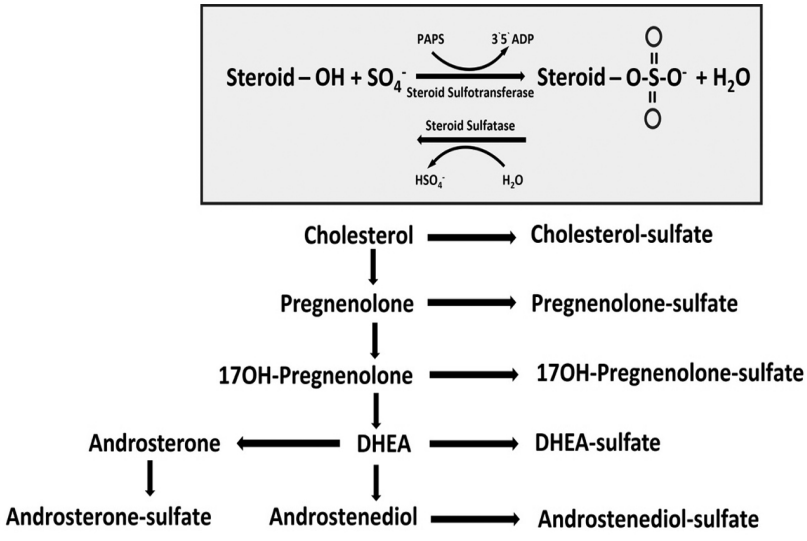

Figure 2. Schematic representation of the implication of steroid sulfatase and steroid sulfotransferase in the regulation of active hydroxylated steroids. The majority of steroid hormone precursors involved in androgen synthesis via canonical or backdoor pathways can be modified by sulfate conjugation

mechanism would also be expected to improve the bone phenotype and would also rescue DHEAS concentrations. Another possibility, at least in some cases with PAPSS2 deficiency, is the existence of at least one currently unknown rescue pathways specific to steroid hormones to prevent androgen excess. Due to the rarity of the disease, multicentre studies are needed to clarify the uncertainties regarding steroid hormone alterations in PAPSS2 deficiency. The research agenda should include comprehensive and integrative plasma and/or urine steroid hormone profile assessment studies, particularly to determine the steroid hormones that are sulfated comparatively in patients with and without androgen excess and the extent to which these steroids are sulfated. Further molecular studies to identify compensatory sulphate donors or sulphate supply pathways may be useful. Additionally, *in vitro/ex vivo* functional studies of identified *PAPSS2* mutations may highlight the impact of given variant on androgen inactivation pattern.

Conclusion

In conclusion, a broad spectrum of bone phenotypes, ranging from brachyolmia to SEMD and severe disproportionate short stature, are the main presenting features of PAPSS2 deficiency. All affected individuals have signs and symptoms related to skeletal dysplasia. The only consistent biochemical markers in affected individuals are low DHEAS and low DHEAS/DHEA ratio. Other adrenal and gonadal androgen concentrations are variable. Most published cases do not have clinical/biochemical hyperandrogenism and the prevalence of the androgen excess phenotype is similar to the general population. Neither the bone phenotype nor the steroid hormone profile correlate with the underlying *PAPSS2* mutation, nor do the skeletal and steroid hormone phenotypes correlate with each other. The reasons for the variability in steroid hormone profiles and androgen excess phenotype warrant further investigation.

Ethics

Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Didem Helvacıoğlu, Tülay Güran.

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References

1. Foster PA, Mueller JW. Sulfation Pathways: Insights into steroid sulfation and desulfation pathways. J Mol Endocrinol 2018;61:271-283. Epub 2018 May 15
2. Fuda H, Shimizu C, Lee YC, Akita H, Strott CA. Characterization and expression of human bifunctional 3'-phosphoadenosine 5'-phosphosulphate synthase isoforms. Biochem J 2002;365:497-504.
3. Schröder E, Gebel L, Eremeev AA, Morgner J, Grum D, Knauer SK, Bayer P, Mueller JW. Human PAPS synthase isoforms are dynamically regulated enzymes with access to nucleus and cytoplasm. PLoS One 2012;7:e29559. Epub 2012 Jan 5
4. Ahmad M, Faiyaz Ul Haque M, Ahmad W, Abbas H, Haque S, Krakow D, Rimoin DL, Lachman RS, Cohn DH. Distinct, autosomal recessive form of spondyloepimetaphyseal dysplasia segregating in an inbred Pakistani kindred. Am J Med Genet 1998;78:468-473.
5. Faiyaz ul Haque M, King LM, Krakow D, Cantor RM, Rusiniak ME, Swank RT, Superti-Furga A, Haque S, Abbas H, Ahmad W, Ahmad M, Cohn DH. Mutations in orthologous genes in human spondyloepimetaphyseal dysplasia and the brachymorphic mouse. Nat Genet 1998;20:157-162.
6. Noordam C, Dhir V, McNelis JC, Schlereth F, Hanley NA, Krone N, Smeitink JA, Smeets R, Sweep FC, Claahsen-van der Grinten HL, Arlt W. Inactivating PAPSS2 mutations in a patient with premature pubarche. N Engl J Med 2009;360:2310-2318.
7. Cortes M, Baria AT, Schwartz NB. Sulfation of chondroitin sulfate proteoglycans is necessary for proper Indian hedgehog signaling in the developing growth plate. Development 2009;136:1697-1706. Epub 2009 Apr 15
8. Unger S, Ferreira CR, Mortier GR, Ali H, Bertola DR, Calder A, Cohn DH, Cormier-Daire V, Girisha KM, Hall C, Krakow D, Makitie O, Mundlos S, Nishimura G, Robertson SP, Savarirayan R, Sillence D, Simon M, Sutton VR, Warman ML, Superti-Furga A. Nosology of genetic skeletal disorders: 2023 revision. Am J Med Genet A 2023;191:1164-1209. Epub 2023 Feb 13
9. Handa A, Tham E, Wang Z, Horemuzova E, Grigelioniene G. Autosomal recessive brachyolmia: early radiological findings. Skeletal Radiol 2016;45:1557-1560. Epub 2016 Aug 21
10. Oostdijk W, Idkowiak J, Mueller JW, House PJ, Taylor AE, O'Reilly MW, Hughes BA, de Vries MC, Kant SG, Santen GW, Verkerk AJ, Uitterlinden AG, Wit JM, Losekoot M, Arlt W. PAPSS2 deficiency causes androgen excess via impaired DHEA sulfation--in vitro and in vivo studies in a family harboring two novel PAPSS2 mutations. J Clin Endocrinol Metab 2015;100:672-680. Epub 2015 Jan 16
11. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol 2018;14:270-284. Epub 2018 Mar 23
12. Rosenfield RL. Normal and Premature Adrenarche. Endocr Rev 2021;42:783-814.
13. Sánchez-Guijo A, Neunzig J, Gerber A, Oji V, Hartmann MF, Schuppe HC, Traupe H, Bernhardt R, Wudy SA. Role of steroid sulfatase in steroid homeostasis and characterization of the sulfated steroid pathway: Evidence from steroid sulfatase deficiency. Mol Cell Endocrinol 2016;437:142-153. Epub 2016 Aug 13
14. Miyake N, Elcioglu NH, Iida A, Isguven P, Dai J, Murakami N, Takamura K, Cho TJ, Kim OH, Hasegawa T, Nagai T, Ohashi H, Nishimura G, Matsumoto N, Ikegawa S. PAPSS2 mutations cause autosomal recessive brachyolmia. J Med Genet 2012;49:533-538. Epub 2012 Jul 11
15. Tüysüz B, Yılmaz S, Gül E, Kolb L, Bilguvar K, Evliyaoğlu O, Günel M. Spondyloepimetaphyseal dysplasia Pakistani type: expansion of the phenotype. Am J Med Genet A 2013;161:1300-1308. Epub 2013 Apr 30
16. Iida A, Simsek-Kiper PÖ, Mizumoto S, Hoshino T, Elcioglu N, Horemuzova E, Geiberger S, Yesil G, Kayserili H, Utine GE, Boduroglu K, Watanabe S, Ohashi H, Alanay Y, Sugahara K, Nishimura G, Ikegawa S. Clinical and radiographic features of the autosomal recessive form of

brachyolmia caused by PAPSS2 mutations. Hum Mutat 2013;34:1381-1386. Epub 2013 Jul 26

17. Alkaya DU, Yılmaz S, Evliyaoğlu O, Bilguvar K, Günel M, Tüysüz B. Hyperandrogenism and Skeletal Dysplasia: Evaluation of 7 Patients with PAPSS2 Gene Mutation. J Clin Res Pediatr Endocrinol 2017;9(Suppl 1):1-31.
18. Eltan M, Yavas Abali Z, Arslan Ates E, Kirkgoz T, Kaygusuz SB, Türkyılmaz A, Bereket A, Turan S, Guran T. Low DHEAS Concentration in a Girl Presenting with Short Stature and Premature Pubarche: A Novel PAPSS2 Gene Mutation. Horm Res Paediatr 2019;92:262-268. Epub 2019 Aug 28
19. Bownass L, Abbs S, Armstrong R, Baujat G, Behzadi G, Berentsen RD, Burren C, Calder A, Cormier-Daire V, Newbury-Ecob R, Foulds N, Juliusson PB, Kant SG, Lefroy H, Mehta SG, Merckoll E, Michot C, Monsell F, Offiah AC, Richards A, Rosendahl K, Rustad CF, Shears D, Tveten K, Wellesley D, Wordsworth P; Deciphering Developmental Disorders Study; Smithson S. PAPSS2-related brachyolmia: Clinical and radiological phenotype in 18 new cases. Am J Med Genet A 2019;179:1884-1894. Epub 2019 Jul 16
20. Cao Y, Guan X, Li S, Wu N, Chen X, Yang T, Yang B, Zhao X. Identification of variants in ACAN and PAPSS2 leading to spondyloepi(meta) physeal dysplasias in four Chinese families. Mol Genet Genomic Med2022;10:e1916. Epub 2022 Mar 9
21. P Perez-Garcia EM, Whalen P, Gurtunca N. Novel Inactivating Homozygous PAPSS2 Mutation in Two Siblings With Disproportionate Short Stature. AACE Clin Case Rep 2022;8:89-92.
22. Mustafa S, Hussain MF, Latif M, Ijaz M, Asif M, Hassan M, Faisal M, Iqbal F. A Missense Mutation (c.1037 G > C, p. R346P) in PAPSS2 Gene Results in Autosomal Recessive form of Brachyolmia Type 1 (Hobaek Form) in A Consanguineous Family. Genes (Basel) 2022;13:2096.

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Cardiovascular Risk Factors in Adolescents with Type 1 Diabetes: Prevalence and Gender Differences

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What is already known on this topic?

Cardiovascular diseases (CVD) are the most important cause of morbidity and mortality in patients with type 1 diabetes (T1D). Children with T1D had similar or higher prevalence of being overweight (OW) or obese (Ob) compared to their healthy peers.

What this study adds?

Girls with T1D are more likely to be OW and Ob and to have CVD risk than boys. Interventions to reduce the risk of CVD in adults with T1D should begin from childhood and be tailored to compensate for gender variations.

Abstract

Objective: Cardiovascular diseases (CVD) are the most important cause of morbidity and mortality in patients with type 1 diabetes (T1D). Children with T1D have a similar or higher prevalence of being overweight (OW) or obese (Ob) compared to healthy peers. The aim of this study was to determine the prevalence of CVD risk factors in children and adolescents with T1D and the impact of obesity and sex differences on these factors.

Methods: Data of patients aged 10-21 years and who had been using intensive insulin therapy with a diagnosis of T1D for at least three years were evaluated. Patients were divided into normal weight (NW), OW and Ob groups based on body mass index percentiles. Risk factors for CVD (obesity, dyslipidemia, hypertension) were compared between groups, and impact of gender was also analyzed.

Results: Data of 365 patients (200 girls, 54.8%), were evaluated. Prevalence of OW/Ob was 25.9% and was significantly higher in girls (30.6% vs 20.1%, $p < 0.001$). Rate of hypertension was highest in OW/Ob girls followed by OW/Ob boys, and similar in NW girls and boys ($p = 0.003$). Mean low density lipoprotein cholesterol (LDL-c) and triglyceride (TG) levels were highest in OW/Ob girls, followed by OW/Ob boys, NW girls and NW boys, respectively ($p < 0.001$ and $p < 0.001$, respectively). Mean high density lipoprotein-cholesterol (HDL-c) levels were similar among groups. Rates of high LDL-c and TG were similar between OW/Ob girls and boys and higher than NW girls, followed by NW boys ($p < 0.001$ and $p < 0.001$, respectively). The rate of low HDL-c was similar in OW/Ob girls and boys, and higher than NW girls, followed by NW boys ($p < 0.001$). Overall, girls were 1.9 times more likely than boys to have two or more risk factors for CVD. Factors associated with risk for CVD in multiple logistic regression analyses were being a girl, followed by higher daily insulin dose, higher hemoglobin A1c, and longer duration of diabetes ($r = 0.856$; $p < 0.001$).

Conclusion: In spite of the increased prevalence for obesity in both sexes, the trend for CVD risk factors was greater in Ob girls, followed by Ob boys and NW girls. Girls with T1D are more likely to be OW/Ob and to have CVD risk than boys, highlighting the need for early intervention and additional studies to elucidate the causes.

Keywords: Overweight, obesity, type 1 diabetes, dyslipidemia, hypertension

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Introduction

Cardiovascular diseases (CVD) are the most important cause of morbidity and mortality in patients with type 1 diabetes (T1D) (1). CVD is more common, occurs earlier, and has a higher mortality rate in patients with T1D than individuals without diabetes (2). It has been reported that the pathogenesis of CVD accelerates in patients with T1D and although CVD rarely presents in childhood, subclinical damage to the cardiovascular system begins in the pediatric age group (3). Preventing CVD by alleviating cardiovascular risk factors is an important treatment goal for patients with T1D.

Obesity in the childhood period is also considered to be an important risk factor for CVD in adulthood (4). Being overweight (OW) and having abdominal adiposity have been associated with atherosclerosis and dyslipidemia (5). Children with T1D had similar or higher prevalence of being OW or obese (Ob) compared to their healthy peers, with rates reaching 25-35% (6-11). The risk of CVD is already high in T1D, and this risk may be increased with Ob. It is observed that Ob-related comorbidities increasingly affect individuals with T1D. Ob individuals with T1D have lower insulin sensitivity than non-Ob patients with T1D, and it has been shown recently that CVD risk factors are increased in children with T1D who are OW or Ob (12). A report of the Exchange study showed a higher prevalence of hypertension and dyslipidemia in Ob patients with T1D than in non-Ob patients (13). A recent study involving children and young adults with T1D showed a higher rate of metabolic syndrome (MS) in OW and Ob individuals (14). Unhealthy behavioral habits contribute to increased cardiovascular risk. Smoking and a sedentary lifestyle are associated with major CVD morbidity and mortality in individuals with T1D (15).

In this study we aimed to determine the prevalence of CVD risk factors in children and adolescents with T1D and the impact of OW/Ob and sex differences on these factors.

Methods

The data of patients aged 10-21 years and who had been using intensive insulin therapy (89% multiple-dose insulin therapy, 11% insulin pump) with a diagnosis of T1D for at least three years were evaluated in this descriptive, cross-sectional study. The diagnosis of T1D was confirmed by the presence of anti-GAD, anti-islet, or anti-insulin antibodies.

Body weights were measured in kilograms using a digital weighing scale (SECA, 769) with a sensitivity of 0.1 kg. Heights were measured in meters with the patients in the standing position using a stadiometer (Harpenden, Holtain Ltd., Crymych, Dyfed, UK) with a sensitivity of 0.1 cm. All the measurements were made by a nurse trained in auxology. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and converted to BMI percentiles for age and sex using the Centers for Disease Control and Prevention (CDC) growth charts from 2000 (CDC: BMI for children and teens, http://www.cdc.gov/nccdphp/dnpa/bmi/bmi-for-age.htm). Those with a BMI <10 percentile were considered as underweight (UW), BMI ≥ 10 and BMI <85 percentile as normal weight (NW), BMI ≥ 85 and BMI <95 percentile as OW, and BMI ≥ 95 percentile as Ob. There were only two patients who were UW and we did not include the underweight group in further analysis in the study, since the number was very small and as we aimed to compare NW versus OW and Ob cases. Since the number of patients in the Ob group was low, when comparing in terms of CVD risk factors regarding genders, the OW and Ob groups were combined and the cases were divided into two groups as NW and combined OW/Ob. At the time of evaluation, the chronological age, body weight, height, BMI, blood pressure, hemoglobin A1c (HbA1c), lipid profile, and daily insulin dose per body weight (IU/kg/day) were noted. The age at diagnosis and duration of diabetes were also recorded. All cases had HbA1c values checked every three months and the HbA1c value used in the study was the average of the four HbA1c values examined in the last year before the evaluation was taken. Insulin dose was defined as the total daily units of insulin divided by the body weight in kilograms and total daily insulin dosage was obtained by randomly selecting three days from the patient records.

Blood pressure was measured with a standardized automatic sphygmomanometer in the right arm with an appropriately sized cuff in the sitting position after 10 minutes of rest, and the average of three measurements was recorded for analysis. For the definition of high blood pressure, the definitions determined by the National High Blood Pressure Education Program study group for children and adolescents were used (16). A mean systolic or diastolic blood pressure above the 95th percentile for age, sex, and height was considered high.

Lipid profiles including triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c) were determined in fasting blood samples. Serum lipid levels were measured by an autoanalyzer (Roche Diagnostics). Lipid values were evaluated according to the recommendations of the American Diabetes Association and the International Diabetes Federation (17,18). LDL-c ≥ 100 mg/dL, TC ≥ 200 mg/dL, and TG ≥ 150 mg/dL were defined as high. HDL-c <40 mg/dL

for 10-16 years old and < 40 mg/dL for boys > 16 years old and < 50 mg/dL for girls > 16 years old was considered low. Dyslipidemia was considered to be an elevation of one or more lipid or lipoprotein levels, or for HDL-c, reduced levels. The presence of at least two risk factors for CVD, such as the presence of OW/Ob (BMI ≥85th percentile), high TG levels, low HDL-c levels, and hypertension were considered as increased cardiovascular risk.

The study was approved by the Hacettepe University Institutional Local Ethics Committee (approval number: 16969557-2202, date: 03.12.2019).

Statistical Analysis

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). The normality of the continuous variables was tested by the Kolmogrov-Smirnow test. Continuous data are described as mean ± standard deviation (SD) and categorical data were described as the number of cases (%). Statistical differences between two independent groups were compared by Student's t-test. The differences among more than two independent groups were analyzed by one-way ANOVA. When the p-value from one-way ANOVA was statistically significant, *post hoc* Bonferroni's corrections were used with either a p-value of 0.0167 or 0.0125 to know which group differ from others.

Clinical and laboratory parameters associated with having two or more risk factors for CVD were first evaluated using univariate analysis. The factors that were significant in the univariate analysis were then evaluated with multivariate logistic regression analysis. Independent variables used in the regression analysis were: gender; age at evaluation; HbA1c level; daily insulin dose; duration of diabetes; and presence of type 2 diabetes in the family. A p-value of less than 0.05 was considered statistically significant.

Results

Three hundred and sixty-five patients (200 girls, 54.8%) who were followed for at least three years with the diagnosis of T1D were included in the study. The mean age of the patients at the time of evaluation was 16.4 ± 3.7 years, and ranged from 10 to 21 years. Mean age at diagnosis was 9.6 ± 3.6 years (range 5-17 years), and mean diabetes duration was 7.0 ± 3.1 years (range 3-14 years). Most (89%) of the patients were using multiple-dose insulin therapy and 11% were using insulin pump therapy. Mean HbA1c was 8.8 ± 2.6%, and mean total daily insulin dose was 1.0 ± 0.3 IU/kg. There was no difference between boys and girls in terms of age at evaluation, HbA1c level, duration of diabetes and daily dose of insulin.

The prevalence of OW, and Ob was 19.3%, and 6.6%, respectively, in the study population (22.6%, and 8% in girls and 15.2%, and 4.9% in boys). The rate of OW and Ob was significantly higher in girls than boys (p < 0.001). The three groups (NW, OW and Ob) were at similar ages and had similar HbA1c levels at the time of the evaluation. BMI-SD score was positively correlated to the duration of diabetes (r = 0.768, p < 0.001). Daily dose of insulin per kg body weight was the highest in the Ob group, followed by the OW and NW group (1.7 ± 0.3 IU/kg/d, 1.3 ± 0.3 IU/kg/d, and 0.9 ± 0.2 IU/kg/d, respectively, p < 0.001). The Ob group had the highest rate of hypertension followed by the OW and NW groups (p < 0.001). Moreover, the Ob group had the highest mean LDL-c, TG and TC levels and the lowest HDL-c levels, followed by the OW and NW groups (p < 0.001, p < 0.001, p < 0.001 and p < 0.001, respectively) (Table 1).

Table 1. Clinical and laboratory characteristics of patients with T1D according to the BMI percentiles

	A Normal weight	B Overweight	C Obese	Overall p	A vs B p	A vs C p	B vs C p
Gender							
Girl	138 (38.0%)	45 (12.4%)	16 (4.4%)	**< 0.001**	**< 0.001***	**< 0.001***	**0.004***
Boy	131 (36.1%)	25 (6.9%)	8 (2.2%)	**< 0.001**	**< 0.001***	**< 0.001***	**0.006***
Age at evaluation (years)	16.3 ± 3.6	16.7 ± 3.4	16.7 ± 2.9	0.415			
HbA1c (%)	8.8 ± 2.5	8.7 ± 1.4	8.5 ± 1.0	0.625			
Insulin dose (IU/kg/day)	0.9 ± 0.2	1.3 ± 0.3	1.7 ± 0.3	**< 0.001**	**< 0.001***	**< 0.001***	**< 0.001***
Duration of diabetes (years)	6.4 ± 2.8	7.9 ± 2.5	10.9 ± 1.6	**< 0.001**	**0.003***	**< 0.001***	**< 0.001***
Total cholesterol (mg/dL)	168.7 ± 33.4	185.2 ± 29.5	245.6 ± 13.0	**< 0.001**	**< 0.001***	**< 0.001***	**< 0.001***
HDL-c (mg/dL)	61.7 ± 13.2	56.1 ± 8.5	47.0 ± 2.9	**< 0.001**	**0.009***	**< 0.001***	**0.003***
LDL-c (mg/dL)	99.4 ± 28.4	124.5 ± 13.0	143.8 ± 8.8	**< 0.001**	**< 0.001***	**< 0.001***	**< 0.001***
TG (mg/dL)	89.6 ± 46.5	112.3 ± 30.6	175.3 ± 12.4	**< 0.001**	**< 0.001***	**< 0.001***	**< 0.001***

*Significant after adjusting for multiple comparisons (Bonferroni's correction; p value for significance 0.0167).
HbA1c: hemoglobin A1c, HDL-c: high density lipoprotein-cholesterol, LDL-c: low density lipoprotein-cholesterol, TG: triglyceride, BMI: body mass index, T1D: type 1 diabetes

Overall, hypertension prevalence was 9.4% in the whole population. The rate of hypertension was highest in OW/Ob girls, followed by OW/Ob boys, and similar in NW girls and boys ($p = 0.003$) (Table 2). Mean LDL-c and TG levels were highest in OW/Ob girls, followed by OW/Ob boys, NW girls and NW boys, respectively ($p < 0.001$ and $p < 0.001$ respectively) (Figure 1, Panel 2, 3 and Supplementary Table 1). The mean HDL-c levels were similar among groups (Figure 1, Panel 1 and Supplementary Table 1). Rates of high LDL-c and TG were similar between OW/Ob girls and boys and higher than in NW girls, followed by NW boys ($p < 0.001$ and $p < 0.001$, respectively) (Table 2). The rate of low HDL-c was similar in OW/Ob girls and boys, and higher than in NW girls, followed by NW boys ($p < 0.001$) (Table 2). The proportion having at least two risk factors for CVD was highest in the OW/Ob girls, followed by OW/Ob boys, NW girls, and lowest in NW boys ($p < 0.001$) (Table 2). Overall, girls were 1.9 times more likely than boys to have two or more risk factors for CVD (37/199 vs 16/164).

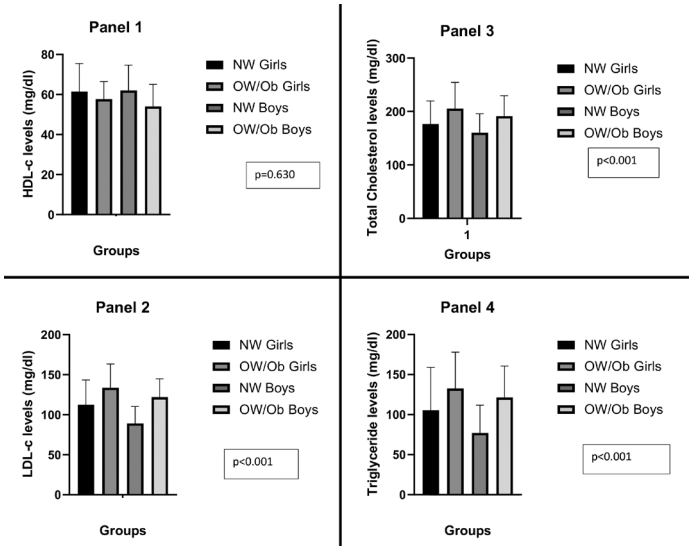

Figure 1. Lipid levels of patients with T1D according to gender and weight status. **Panel 1:** HDL-c levels: There was no significant difference among four groups. **Panel 2:** LDL-c levels: NW Girls vs OW/Ob Girls < 0.0125, NW Girls vs NW Boys < 0.0125, NW Girls vs OW/Ob Boys < 0.0125, OW/Ob Girls vs NW Boys < 0.0125, OW/Ob Girls vs OW/Ob Boys < 0.0125, NW Boys vs OW/Ob Boys < 0.0125. **Panel 3:** Total cholesterol levels: NW Girls vs OW/Ob Girls < 0.0125, NW Girls vs NW Boys < 0.0125, NW Girls vs OW/Ob Boys < 0.0125, OW/Ob Girls vs NW Boys < 0.0125, OW/Ob Girls vs OW/Ob Boys < 0.0125, NW Boys vs OW/Ob Boys < 0.0125. **Panel 4:** TG levels: NW Girls vs OW/Ob Girls < 0.0125, NW Girls vs NW Boys < 0.0125, NW Girls vs OW/Ob Boys < 0.0125, OW/Ob Girls vs NW Boys < 0.0125, OW/Ob Girls vs OW/Ob Boys < 0.0125, NW Boys vs OW/Ob Boys < 0.0125

NW: normal weight, OW: overweight, Ob: obese, HDL-c: high density lipoprotein-cholesterol, LDL-c: low density lipoprotein-cholesterol

The patients with two or more risk factors for CVD were older, had higher HbA1c levels, were using a higher dose of daily insulin per kg of body weight, and had longer duration of diabetes in comparison to those with a low risk for CVD which persisted in gender specific analyses (Table 3).

Factors associated with risk for CVD in multiple logistic regression analyses were being a girl, followed by higher daily insulin dose, higher HbA1c, and longer diabetes duration ($r = 0.856$; $p < 0.001$) (Table 4).

Discussion

The prevalence of obesity in children and adolescents with T1D is increasing, in parallel with the general population (6,19,20). One in four T1D patients in this study had a BMI over the 85th percentile, which is a higher prevalence than that of healthy Turkish children (21,22,23). Although there are no nationwide statistics on the frequency of Ob in children and adolescents with T1D, a few regional studies also found a similar prevalence (24,25). Previous research from various regions of the world revealed that 25-38.5% of children with T1D were either OW or Ob (6,8,9). According to the Diabetes Control and Complications and The Epidemiology of Diabetes Interventions and Complications Trials (EDIC), intensive insulin treatment, that is multiple insulin injections and insulin pumps, may be responsible for the increased prevalence of Ob in T1D patients compared to the non-diabetic population (26).

In the present study, girls were 1.5 times more likely to be OW/Ob than boys (30.6% vs 20.1%), whereas this is more common in boys in the non-diabetic pediatric population (21,27). There are similar studies showing that girls with T1D are particularly vulnerable to become OW/Ob, particularly during puberty (11,28). It is not known exactly why the obesity rate is higher in girls with T1D although insulin resistance, gender-specific hormonal differences, changes in body composition and energy metabolism during puberty in girls and boys may all contribute to the observed sex-related disparities in prevalence of OW/Ob in children and adolescents with T1D (14,29).

The most common cause of death in T1D is CVD. The prevalence of CVD-related and all-cause mortality is ten times higher in people with T1D than in the general population, despite recent advances in glycemic control and CVD risk management (30). The cumulative incidence of coronary artery disease by age 55 is as high as 35% in T1D compared to <10% in the non-diabetic population. Risk of stroke is four times higher in T1D compared to non-diabetic population. Similarly, people with T1D have a five-fold higher risk and incidence of peripheral vascular disease

Table 2. Clinical and laboratory characteristics of the patients with T1D according to BMI percentiles and gender

	A NW Girls (n = 38)	B OW/Ob Girls (n = 61)	C NW Boys (n = 131)	D OW/Ob Boys (n = 33)	Overall p value	A vs B p	A vs C p	A vs D p	B vs C p	B vs D p	C vs D p
Age at evaluation (yrs)	16.2 ± 2.7	16.6 ± 3.8	16.4 ± 3.7	16.9 ± 3.5	0.498						
HbA1c (%)	8.7 ± 1.6	8.5 ± 1.0	8.9 ± 3.1	8.6 ± 1.8	0.649						
Hypertension	5.8% (8/138)	21.3% (13/61)	6.1% (8/131)	15.2% (5/33)	**0.003**	**< 0.001***	**0.716**	**< 0.001***	**< 0.001***	**0.008***	**< 0.001***
TG ≥150 mg/dL	14.5% (20/138)	34.4% (21/61)	6.1% (8/131)	27.3% (9/33)	**< 0.001**	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	0.020	**< 0.001***
HDL-c < 40 mg/dL (girls > 16 years of age < 50 mg/dL)	11.6% (16/138)	21.3% (13/61)	6.1% (8/131)	18.2% (6/33)	**< 0.001**	**< 0.001***	**0.003***	**0.005***	**< 0.001***	0.678	**< 0.001**
LDL-c ≥100 mg/dL	36.2% (50/138)	62.3% (38/61)	22.9% (30/131)	54.6% (18/33)	**< 0.001**	**< 0.001***	**0.006***	**0.002***	**< 0.001***	0.392	**< 0.001***
Dyslipidemia	36.2% (50/138)	62.3% (38/61)	22.9% (30/131)	54.6% (18/33)		**< 0.001***	**0.006***	**0.002***	**< 0.001***	0.392	**< 0.001***
At least two criterias positivity for MS [obesity and/or TG ≥150 mg/dL and/or HDL < 40 mg/dL (girls > 16 years of age < 50 mg/dL) and/or hypertension]	11.6% (16/138)	34.4% (21/61)	5.3% (7/131)	27.3% (9/33)	**< 0.001**	**< 0.001***	**0.002***	**< 0.001***	**< 0.001***	**0.009***	**< 0.001***

*Significant after adjusting for multiple comparisons (Bonferroni's correction; p value for significance 0.0125).
yrs: years, HbA1c: hemoglobin A1c, HDL-c: high density lipoprotein-cholesterol, LDL-c: low density lipoprotein-cholesterol, TG: triglyceride, BMI: body mass index, T1D: type 1 diabetes, MS: metabolic syndrome, NW: normal weight, OW: overweight, Ob: obese

Table 3. Comparison of patients according to the presence of increased cardiovascular risk

	All patients		p	Girls		p	Boys		p
	CVD risk (+)	CVD risk (-)		CVD risk (+)	CVD risk (-)		CVD risk (+)	CVD risk (-)	
Age at evaluation (yrs)	18.2 ± 2.5	16.1 ± 2.7	**< 0.001**	18.1 ± 2.5	15.9 ± 2.3	**< 0.001**	18.6 ± 2.5	16.3 ± 3.0	**< 0.001**
Gender			**< 0.001**						
Girls	37 (69.8%)	162 (52.3%)							
Boys	16 (39.2%)	148 (47.7%)							
HbA1c (%)	9.7 ± 1.4	8.6 ± 1.9	**< 0.001**	9.5 ± 1.0	8.5 ± 1.2	**< 0.001**	10.3 ± 1.9	8.7 ± 2.4	**< 0.001**
Insulin dose (IU/kg/day)	1.4 ± 0.3	1.0 ± 0.3	**< 0.001**	1.5 ± 0.3	1.1 ± 0.2	**< 0.001**	1.3 ± 0.2	0.9 ± 0.3	**< 0.001**
Diabetes duration (yrs)	9.7 ± 1.5	6.6 ± 2.6	**< 0.001**	9.9 ± 1.9	6.6 ± 2.3	**< 0.001**	9.1 ± 2.8	6.5 ± 2.3	**< 0.001**

yrs: years, CVD: cardiovascular diseases, HbA1c: hemoglobin A1c

Table 4. Factors affecting having two or more risk factors for CVD based on multivariate regression analysis

Variables	Standardized beta coefficient	Odds ratio	95% CI		p
			Lowest	Highest	
Being a girl	5.605	11.483	6.480	18.095	**< 0.001**
Longer diabetes duration	0.832	1.137	1.033	1.573	**0.04**
Higher HbA1c	0.980	2.633	1.179	12.011	**0.03**
Higher daily insulin dose	1.036	3.409	1.695	8.714	**< 0.001**

CI: confidence interval, CVD: cardiovascular diseases, HbA1c: hemoglobin A1c

than people without diabetes. Atherosclerosis begins in childhood and is more common in children and adolescents with T1D, as shown in autopsy and epidemiological studies (31,32). Obesity may have a further impact on the risk of CVD associated with T1D. The EDIC study reported a significant increase in lipid levels and blood pressure in subjects with excessive weight gain while using intensive insulin therapy, similar to insulin resistance syndromes (26). The results of the present study showed that CVD risk factors in OW/Ob diabetic adolescents were more prevalent than that of normal-weight adolescents with T1D. We also showed that girls are more likely to have risk factors for CVD in comparison to boys in a T1D population.

Hypertension is also more common in patients with T1D than in the general population (33). Risk factors for hypertension in adolescents with T1D include obesity and hyperglycemia (34). In the current study the overall prevalence of hypertension was similar to the figures reported in previous studies (35). However, we showed that hypertension was more common in Ob girls with T1D; this is in contrast to the prevalence in the Ob non-diabetic population where hypertension is more common in boys than in girls (36,37). However, it should be noted that this difference (21% vs 15%, $p = 0.008$) was less remarkable than the difference between NW and the Ob diabetic population (5-6% vs 15-21%, $p < 0.001$).

Dyslipidemia is recognized as one of the most important CVD risk factor in patients with diabetes (38). Poor metabolic control causes diabetic dyslipidemia, which is characterized by increased LDL-c and TG levels and low HDL-c levels (39,40). The EDIC study showed that elevated LDL-c and low HDL-c lasting more than 10 years were associated with a higher CVD risk in the T1D population (41). Diabetes is a disease that causes accelerated atherosclerosis and therefore requires regular lipid monitoring and early intervention (42). Most studies showed that 26-58% of children with T1D have lipid levels above these defined values (43,44,45). Adolescent girls with T1D were reported to have higher mean TG, LDL-c, and Apolipoprotein B levels than boys, although HbA1c levels were not different from boys, suggesting that girls with T1D are at risk for CVD starting from younger ages (45). In the current study, the mean LDL-c and TG were greater in Ob girls than in Ob boys, however the prevalence of increased LDL-c and TG as CVD risk factors was similar in both Ob boys and girls with T1D and was significantly higher than that of NW girls with T1D followed by that of NW boys with T1D. In addition, similar to other studies, there was no difference in HbA1c values between the four groups. Despite comparable mean levels across all groups, prevalence of low HDL-c as a risk factor was associated with similar variation in the current study. These findings suggest that obesity plays a significant role in inducing dyslipidemia, and that female sex may also be a secondary risk factor.

A recent study showed that the rate of MS was higher in OW (8.1%) and Ob (35.3%) cases than NW (4.9%) in a population of children, adolescents and young adults with T1D (14). These rates were higher than those in the non-diabetic population where it is reported to have a median prevalence of 11.9% (range 2.8-29.3%) and 29.2% (range 10-66%) in OW and Ob children, respectively (46). The prevalence of MS (0-1%) is even lower in NW, non-diabetic children (46). In children and adolescents, a limited number of studies report a gender predilection for MS, which is mostly in favor of boys (36). However, this predilection does not seem to be preserved in patients with T1D (47,48).

The presence of at least two risk factors for CVD, such as the presence of obesity (BMI ≥85 percentile), high TG levels, low HDL-c levels, and hypertension were considered as increased cardiovascular risk in the current study. In the current study, a high proportion of OW/Ob adolescents with T1D also had other CVD risk factors including elevated TGs (31.9%), elevated blood pressure (19.1%) and low HDL-c (20.2%). Similar to our study, different studies have shown that the risk of hypertension and dyslipidemia increases with an increase in BMI and the majority of Ob children with T1D had at least two CVD risk factors (13,49). This is important because the coexistence of multiple CVD risk factors increases the risk of morbidity and mortality. Having a higher number of CVD risk factors is associated with more progressive early atherosclerotic lesions (50,51).

In the current study, approximately 20% of the girls and 10% of the boys with T1D had at least two CVD risk factors. Girls were twice as likely to have a CVD risk factor as boys. In addition to obesity, being female appears to be a risk factor in terms of CVD. In the regression analysis the most important factor increasing the CVD risk factor was being a girl. In Ob girls, the presence of CVD risk factors was approximately three times more common than in normal-weight girls and 1.3 times more common than in Ob boys (34.4% vs 11.6%, and 34.4% vs 27.3%, respectively). Brown et al. (52) showed that TC, LDL-c, BMI and blood pressure were higher in adolescent girls than boys, similar to our findings. The prevalence of co-existence of several CVD risk factors has been reported to be more common in girls than in boys (40,53,54,55).

Given the later onset of CVD in women in the general population, premenopausal women appear to be protected from CVD compared to men of similar age.

These differences among genders have been attributed to biological differences, including sex chromosomes and hormonal status, as well as to gender differences in behavioral and sociocultural aspects (56). Considering that the risk of CVD increases as estrogen levels decrease after menopause, estrogens appear to be protective against CVD. By increasing the amount of HDL-c and inhibiting LDL-c oxidation, estrogen in the blood causes a lipoprotein profile change, which reduces the accumulation of oxidized LDL-c in the arterial wall (57). Gender-related protection from CVD is lost in females with diabetes. Females with T1D have a roughly 40% greater risk of all-cause death and twice the risk of fatal and non-fatal vascular events compared to males with T1D (58). It is not fully explained why the CVD risk is higher in females with T1D compared to males. The mechanisms proposed to date argue for gender differences in both biological and psychosocial factors, as well as in the management of diabetes and CVD risk factors (35). Hormonal changes seen in women with T1D may contribute to a greater atherogenic lipid profile, insulin resistance, higher inflammation, and loss of vasoprotective effect (59). This is a mechanism that warrants further investigation. It was also thought that the higher prevalence of OW/Ob and CVD risk in adolescent girls with T1D might be related to the difficulties encountered in the management of T1D in this population. Sedentary lifestyle and physical inactivity, which are among the leading modifiable risk factors for CVD, are more common in girls with T1D compared to boys (59).

There is also substantial evidence that insulin resistance in T1D may diminish the female protection against CVD. The CACTI study has shown that adults with T1D have increased insulin resistance compared to nondiabetic controls (60) and, more recently, that this effect of T1D on insulin resistance appears to be greater in women (61). The reasons for greater insulin resistance in women with T1D compared to women without diabetes are unclear, but it is thought that differences in estrogen levels may play a role. It has been also shown that healthy girls are less insulin sensitive than boys, but compensate for their decreased sensitivity with increased insulin secretion (62). However, this compensation does not exist in diabetic children and adolescents.

It is also not clear when excess CVD risk begins in women with T1D. We have shown that sex differences in the prevalence of CVD risk factors emerge in the early course of T1D, beginning with adolescence. In particular, adolescent girls with T1D have higher CVD risk factors than boys. Therefore, early diagnosis and gender-specific intervention in girls and young women with T1D will facilitate earlier reduction of the risk of CVD with its attendant morbidity and mortality later in life.

Study Limitations

One of the strengths of this study was the large sample size of children and adolescents with a long follow-up period from a single center. Participants in the study were exposed to comparable management strategies, which is an strength. However, this study also has some limitations. First, since data were gathered retrospectively, measurements such as waist circumference were absent, despite the fact that it is optimal to evaluate obesity using waist circumference in children and adolescents. Second, some potential confounders of obesity and CVD risk, including levels of physical activity, dietary practice, and socioeconomic status, were not accounted for. Thirdly, this was not a longitudinal study, nor was it a population-based study and thus the prevalence rates are not applicable to the entire population of children and adolescents with T1D. The last limitation was that we did not compare our patients to a control group of non-diabetic children.

Conclusion

This study showed that more than a quarter of children and adolescents with T1D were OW or Ob. In spite of the increased prevalence for obesity in both sexes, the trend for CVD risk factors was increased most in Ob girls, followed by Ob boys and then girls who were of NW.

Girls with T1D are more likely to be OW and Ob and to have CVD risk factors than boys, highlighting the need for early intervention and additional studies to elucidate the causes. Interventions to reduce the risk of CVD in adults with T1D should begin from childhood and be tailored to compensate for gender variations. Individuals with T1D may experience less CVD morbidity and mortality as a result of early identification of CVD risk factors and possible gender-specific treatments, which may ultimately lead to better long-term outcomes.

Ethics

Ethics Committee Approval: The study was approved by the Hacettepe University Institutional Local Ethics Committee (approval number: 16969557-2202, date: 03.12.2019).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: Doğuş Vurallı, Z. Alev Özön, E. Nazlı Gönç, Ayfer Alikaşifoğlu, Nurgün Kandemir, Concept: Doğuş Vurallı, Nurgün Kandemir, Design: Doğuş

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References

1. Rawshani A, Rawshani A, Gudbjörnsdottir S. Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes. N Engl J Med 2017;377:300-301.
2. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. Diabetes Care 2006;29:798-804.
3. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. Circulation 2004;109:1750-1755. Epub 2004 Mar 15
4. Park MH, Falconer C, Viner RM, Kinra S. The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. Obes Rev 2012;13:985-1000. Epub 2012 Jun 26
5. Valerio G, Iafusco D, Zucchini S, Maffeis C; Study-Group on Diabetes of Italian Society of Pediatric Endocrinology and Diabetology (ISPED). Abdominal adiposity and cardiovascular risk factors in adolescents with type 1 diabetes. Diabetes Res Clin Pract 2012;97:99-104. Epub 2012 Feb 13
6. Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, Pihoker C, Dabelea D, Hamman R, Waitzfelder B, Kahn HS; SEARCH for Diabetes in Youth Study Group. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. Pediatr Diabetes 2010;11:4-11. Epub 2009 May 15
7. Islam ST, Abraham A, Donaghue KC, Chan AK, Lloyd M, Srinivasan S, Craig ME. Plateau of adiposity in Australian children diagnosed with Type 1 diabetes: a 20-year study. Diabet Med 2014;31:686-690. Epub 2014 Mar 6
8. Baskaran C, Volkening LK, Diaz M, Laffel LM. A decade of temporal trends in overweight/obesity in youth with type 1 diabetes after the Diabetes Control and Complications Trial. Pediatr Diabetes 2015;16:263-270. Epub 2014 Jul 12
9. Fröhlich-Reiterer EE, Rosenbauer J, Bechtold-Dalla Pozza S, Hofer SE, Schober E, Holl RW; DPV-Wiss Study Group and German BMBF Competence Networks Diabetes mellitus and Obesity. Predictors of increasing BMI during the course of diabetes in children and adolescents with type 1 diabetes: data from the German/Austrian DPV multicentre survey. Arch Dis Child 2014;99:738-743. Epub 2014 May 8
10. Kapellen TM, Gausche R, Dost A, Wiegand S, Flechtner-Mors M, Keller E, Kiess W, Holl RW; DPV-Science-Initiative of the German Competence Network Diabetes and the German Competence Network Obesity (Consortium LARGE). Children and adolescents with type 1 diabetes in Germany are more overweight than healthy controls: results comparing DPV database and CrescNet database. J Pediatr Endocrinol Metab 2014;27:209-214.
11. Holl RW, Grabert M, Sorgo W, Heinze E, Debatin KM. Contributions of age, gender and insulin administration to weight gain in subjects with IDDM. Diabetologia1998;41:542-547.
12. Maahs DM, Daniels SR, de Ferranti SD, Dichek HL, Flynn J, Goldstein BI, Kelly AS, Nadeau KJ, Martyn-Nemeth P, Osganian SK, Quinn L, Shah AS, Urbina E; American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular and Stroke Nursing, Council for High Blood Pressure Research, and Council on Lifestyle and Cardiometabolic Health. Cardiovascular disease risk factors in youth with diabetes mellitus: a scientific statement from the American Heart Association. Circulation 2014;130:1532-1558. Epub 2014 Aug 28
13. Redondo MJ, Foster NC, Libman IM, Mehta SN, Hathway JM, Bethin KE, Nathan BM, Ecker MA, Shah AC, DuBose SN, Tamborlane WV, Hoffman RP, Wong JC, Maahs DM, Beck RW, DiMeglio LA. Prevalence of cardiovascular risk factors in youth with type 1 diabetes and elevated body mass index. Acta Diabetol 2016;53:271-277. Epub 2015 Jun 16
14. Pinhas-Hamiel O, Levek-Motola N, Kaidar K, Boyko V, Tisch E, Mazor-Aronovitch K, Graf-Barel C, Landau Z, Lerner-Geva L, Frumkin Ben-David R. Prevalence of overweight, obesity and metabolic syndrome components in children, adolescents and young adults with type 1 diabetes mellitus. Diabetes Metab Res Rev 2015;31:76-84.
15. Donaghue K, Jeanne Wong SL. Traditional Cardiovascular Risk Factors in Adolescents with Type 1 Diabetes Mellitus. Curr Diabetes Rev 2017;13:533-543.
16. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(2 Suppl 4th Report):555-576.
17. American Diabetes Association. Standards of medical care in diabetes--2012. Diabetes Care 2012;35(Suppl 1):11-63.
18. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, Wong G, Bennett P, Shaw J, Caprio S; IDF Consensus Group. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr Diabetes 2007;8:299-306.
19. Maffeis C, Birkebaek NH, Konstantinova M, Schwandt A, Vazeou A, Casteels K, Jali S, Limbert C, Pundziute-Lycka A, Toth-Heyn P, de Beaufort C, Sumnik Z, Cherubini V, Svensson J, Pacaud D, Kanaka-Gantenbein C, Shalitin S, Bratina N, Hanas R, Alonso GT, Poran L, Pereira AL, Marigliano M; SWEET Study Group. Prevalence of underweight, overweight, and obesity in children and adolescents with type 1 diabetes: Data from the international SWEET registry. Pediatr Diabetes 2018;19:1211-1220. Epub 2018 Aug 22
20. Łuczyński W, Szypowska A, Głowińska-Olszewska B, Bossowski A. Overweight, obesity and features of metabolic syndrome in children with diabetes treated with insulin pump therapy. Eur J Pediatr 2011;170:891-898. Epub 2010 Dec 8
21. Çelmeli G, Çürek Y, Arslan Gülten Z, Yardımsever M, Koyun M, Akçurin S, Bircan İ. Remarkable Increase in the Prevalence of Overweight and Obesity Among School Age Children in Antalya, Turkey, Between 2003 and 2015. J Clin Res Pediatr Endocrinol 2019;11:76-81. Epub 2018 Sep 25
22. Deniz S, Oguzoncul AF. The prevalence of obesıty and related factors among prımary and secondary school students. Niger J Clin Pract 2019;22:1685-1692.
23. Alper Z, Ercan İ, Uncu Y. A Meta-Analysis and an Evaluation of Trends in Obesity Prevalence among Children and Adolescents in Turkey: 1990 through 2015. J Clin Res Pediatr Endocrinol 2018;10:59-67. Epub 2017 Sep 13

24. Yayıcı Köken Ö, Kara C, Can Yılmaz G, Aydın HM. Prevalence of Obesity and Metabolic Syndrome in Children with Type 1 Diabetes: A Comparative Assessment Based on Criteria Established by the International Diabetes Federation, World Health Organisation and National Cholesterol Education Program. J Clin Res Pediatr Endocrinol 2020;12:55-62. Epub 2019 Aug 22

25. Özkaya V, Eren E, Özgen Özkaya Ş, Denkboy Öngen Y. Prevalence of obesity in prepubertal and pubertal with Turkish population type 1 diabetes. Diabetol Int 2021;12:412-419.

26. Purnell JQ, Zinman B, Brunzell JD; DCCT/EDIC Research Group. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) study. Circulation 2013;127:180-187.

27. Lobstein T BH. Atlas of childhood obesity. World Obesity Federation, 2019.

28. Ingberg CM, Särnblad S, Palmér M, Schvarcz E, Berne C, Aman J. Body composition in adolescent girls with type 1 diabetes. Diabet Med 2003;20:1005-1011.

29. Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999;48:2039-2044.

30. Colom C, Rull A, Sanchez-Quesada JL, Pérez A. Cardiovascular Disease in Type 1 Diabetes Mellitus: Epidemiology and Management of Cardiovascular Risk. J Clin Med 2021;10:1798.

31. Järvisalo MJ, Putto-Laurila A, Jartti L, Lehtimäki T, Solakivi T, Rönnemaa T, Raitakari OT. Carotid artery intima-media thickness in children with type 1 diabetes. Diabetes 2002;51:493-498.

32. Krantz JS, Mack WJ, Hodis HN, Liu CR, Liu CH, Kaufman FR. Early onset of subclinical atherosclerosis in young persons with type 1 diabetes. J Pediatr 2004;145:452-457.

33. Ahmadizar F, Souverein P, de Boer A, Maitland-van der Zee AH. Undertreatment of hypertension and hypercholesterolaemia in children and adolescents with type 1 diabetes: long-term follow-up on time trends in the occurrence of cardiovascular disease, risk factors and medications use. Br J Clin Pharmacol 2018;84:776-785. Epub 2018 Jan 25

34. Koebnick C, Imperatore G, Jensen ET, Stafford JM, Shah AS, Mottl AK, Bell RA, Dabelea D, Liese AD, Marcovina SM, D'Agostino RB Jr, Urbina EM, Lawrence JM. Progression to hypertension in youth and young adults with type 1 or type 2 diabetes: The SEARCH for Diabetes in Youth Study. J Clin Hypertens (Greenwich) 2020;22:888-896. Epub 2020 Apr 15

35. Downie ML, Ulrich EH, Noone DG. An Update on Hypertension in Children With Type 1 Diabetes. Can J Diabetes 2018;42:199-204. Epub 2018 Mar 6

36. Barstad LH, Júlíusson PB, Johnson LK, Hertel JK, Lekhal S, Hjelmesæth J. Gender-related differences in cardiometabolic risk factors and lifestyle behaviors in treatment-seeking adolescents with severe obesity. BMC Pediatr 2018;18:61.

37. Daratha KB, Bindler RC. Effects of individual components, time, and sex on prevalence of metabolic syndrome in adolescents. Arch Pediatr Adolesc Med 2009;163:365-370.

38. Hero C, Rawshani A, Svensson AM, Franzén S, Eliasson B, Eeg-Olofsson K, Gudbjörnsdottir S. Association Between Use of Lipid-Lowering Therapy and Cardiovascular Diseases and Death in Individuals With Type 1 Diabetes. Diabetes Care 2016;39:996-1003. Epub 2016 Apr 18

39. Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes Ther 2016;7:203-219. Epub 2016 Apr 7

40. Schwab KO, Doerfer J, Marg W, Schober E, Holl RW; DPV Science Initiative and the Competence Network Diabetes mellitus. Characterization of 33 488 children and adolescents with type 1 diabetes based on the gender-specific increase of cardiovascular risk factors. Pediatr Diabetes 2010;11:357-363.

41. Orchard TJ, Forrest KY, Kuller LH, Becker DJ; Pittsburgh Epidemiology of Diabetes Complications Study. Lipid and blood pressure treatment goals for type 1 diabetes: 10-year incidence data from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Care 2001;24:1053-1059.

42. Glaser NS, Geller DH, Haqq A, Gitelman S, Malloy M; Lawson Wilkins Pediatric Endocrine Society Committee on Drugs and Therapeutics. Detecting and treating hyperlipidemia in children with type 1 diabetes mellitus: are standard guidelines applicable to this special population? Pediatr Diabetes 2011;12:442-459. Epub 2010 Nov 5

43. Reh CM, Mittelman SD, Wee CP, Shah AC, Kaufman FR, Wood JR. A longitudinal assessment of lipids in youth with type 1 diabetes. Pediatr Diabetes 2011;12:365-371. Epub 2011 Mar 11

44. Kershnar AK, Daniels SR, Imperatore G, Palla SL, Petitti DB, Pettitt DJ, Marcovina S, Dolan LM, Hamman RF, Liese AD, Pihoker C, Rodriguez BL. Lipid abnormalities are prevalent in youth with type 1 and type 2 diabetes: the SEARCH for Diabetes in Youth Study. J Pediatr 2006;149:314-319.

45. Macedoni M, Hovnik T, Plesnik E, Kotnik P, Bratina N, Battelino T, Groselj U. Metabolic control, ApoE genotypes, and dyslipidemia in children, adolescents and young adults with type 1 diabetes. Atherosclerosis 2018;273:53-58. Epub 2018 Apr 12

46. Al-Hamad D, Raman V. Metabolic syndrome in children and adolescents. Transl Pediatr 2017;6:397-407.

47. Harjutsalo V, Maric-Bilkan C, Forsblom C, Groop PH. Impact of sex and age at onset of diabetes on mortality from ischemic heart disease in patients with type 1 diabetes. Diabetes Care 2014;37:144-148. Epub 2013 Sep 23

48. Laing SP, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR, Gatling W, Bingley PJ, Patterson CC. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 2003;46:760-765. Epub 2003 May 28

49. van Vliet M, Van der Heyden JC, Diamant M, Von Rosenstiel IA, Schindhelm RK, Aanstoot HJ, Veeze HJ. Overweight is highly prevalent in children with type 1 diabetes and associates with cardiometabolic risk. J Pediatr 2010;156:923-929. Epub 2010 Mar 10

50. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-952.

51. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-1656.

52. Brown TL, Maahs DM, Bishop FK, Snell-Bergeon JK, Wadwa RP. Influences of gender on cardiovascular disease risk factors in adolescents with and without type 1 diabetes. Int J Pediatr Endocrinol 2016;2016:8. Epub 2016 Apr 19

53. Schwab KO, Doerfer J, Hecker W, Grulich-Henn J, Wiemann D, Kordonouri O, Beyer P, Holl RW; DPV Initiative of the German Working Group for Pediatric Diabetology. Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type 1 diabetes: cross-sectional data from the German diabetes documentation and quality management system (DPV). Diabetes Care 2006;29:218-225.

54. Margeirsdottir HD, Larsen JR, Brunborg C, Overby NC, Dahl-Jørgensen K; Norwegian Study Group for Childhood Diabetes. High prevalence of cardiovascular risk factors in children and adolescents with type 1 diabetes: a population-based study. Diabetologia 2008;51:554-561. Epub 2008 Jan 15

55. MacKenzie KE, Wiltshire EJ, Peña AS, Gent R, Hirte C, Piotto L, Couper JJ. Hs-CRP is associated with weight, BMI, and female sex but not with endothelial function in children with type 1 diabetes. Pediatr Diabetes 2009;10:44-51. Epub 2008 Sep 15

56. EUGenMed Cardiovascular Clinical Study Group; Regitz-Zagrosek V, Oertelt-Prigione S, Prescott E, Franconi F, Gerdts E, Foryst-Ludwig A, Maas AH, Kautzky-Willer A, Knappe-Wegner D, Kintscher U, Ladwig KH, Schenck-Gustafsson K, Stangl V. Gender in cardiovascular diseases: impact on clinical manifestations, management, and outcomes. Eur Heart J 2016;37:24-34. Epub 2015 Nov 3

57. Hsu SP, Lee WS. Effects of female sex hormones on the development of atherosclerosis. Chin J Physiol 2020;63:256-262.

58. Huxley RR, Peters SA, Mishra GD, Woodward M. Risk of all-cause mortality and vascular events in women versus men with type 1 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol 2015;3:198-206. Epub 2015 Feb 6

59. Smigoc Schweiger D, Battelino T, Groselj U. Sex-Related Differences in Cardiovascular Disease Risk Profile in Children and Adolescents with Type 1 Diabetes. Int J Mol Sci 2021;22:10192.

60. Schauer IE, Snell-Bergeon JK, Bergman BC, Maahs DM, Kretowski A, Eckel RH, Rewers M. Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study. Diabetes 2011;60:306-314. Epub 2010 Oct 26

61. Millstein RJ, Pyle LL, Bergman BC, Eckel RH, Maahs DM, Rewers MJ, Schauer IE, Snell-Bergeon JK. Sex-specific differences in insulin resistance in type 1 diabetes: The CACTI cohort. J Diabetes Complications 2018;32:418-423. Epub 2018 Jan 10

62. Hoffman RP, Vicini P, Sivitz WI, Cobelli C. Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. Pediatr Res 2000;48:384-388.

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ORIGINAL ARTICLE

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Association of Vitamin D Deficiency and Vitamin D Receptor Gene Polymorphisms with Type 1 Diabetes Risk: A South Indian Familial Study

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What is already known on this topic?

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by the depletion of pancreatic β-cells, hypoglycemia, and elevated inflammatory cytokines in the case of serum vitamin D deficiency (VDD). Vitamin D receptor (VDR) haplotype polymorphisms are associated with T1D in several population.

What this study adds?

The genotype frequency of *VDR* gene polymorphisms FokI (rs2228570 C/T)-FF is significantly higher in T1D patients than controls and this relationship is reversed in the FokI-Ff genotype. VDD appears to be contributing risk factor to T1D development in South Indian children.

Abstract

Objective: Vitamin D is a potent immune modulator and is associated with autoimmune diseases, including type 1 diabetes (T1D). The vitamin D levels and its receptor gene polymorphisms together in T1D are not yet investigated in the South Indian population. The present study focused on exploring the significance of vitamin D levels and vitamin D receptor (VDR) gene polymorphisms with the risk of developing T1D in the South Indian population.

Methods: Patients with T1D and unaffected first-degree relatives (FDRs) were included in this study. Genotyping of VDR polymorphisms at four different loci (FokI- F/f, BsmI- B/b, TaqI- T/t, and ApaI- A/a) was assessed through the amplification refractive mutation system-polymerase chain reaction method. Serum vitamin D levels were measured in 98 T1D patients and 75 age- and sex-matched siblings.

Results: A total of 120 patients with T1D and 214 FDRs were included. Vitamin D deficiency (VDD) was observed in a higher proportion of T1D patients than in controls (52% vs. 32%; $p < 0.03$). The frequency of the FokI-FF genotype was significantly higher [odds ratio (OR) = 1.66; $p < 0.03$] in T1D patients conferring a susceptible association with the disease. Nevertheless, the increased frequency of heterozygous Ff genotype (OR = 0.57; $p < 0.02$) among controls may confer a protective association with T1D. Furthermore, the transmission disequilibrium test revealed over-transmission of ApaI-A (T: U = 15/5; $p < 0.006$) and BsmI-B alleles (T: U = 17/5; $p < 0.01$) and under-transmission of BsmI-b/ApaI-a/TaqI-T haplotype (T: U = 5.4/14.4; $p = 0.04$) from parents to T1D patients.

Conclusion: The present study concludes that VDD is the major contributing risk factor to T1D development in the South Indian population. Furthermore, the FokI-FF genotype, BsmI-B, and ApaI-A alleles were positively associated with T1D. In contrast, the FokI-Ff genotype and BsmI-b/ApaI-a/TaqI-T haplotype were negatively associated with T1D.

Keywords: Vitamin D, type 1 diabetes, autoimmunity, polymorphism, vitamin D receptor, β-cells

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Introduction

Type 1 diabetes mellitus (T1D) is characterized by the destruction of insulin-producing β-cells of pancreatic islets due to aberrations in both humoral and cell-mediated immunity (1,2). As a result of this autoimmune process, the pancreas produces very little or no insulin, which leads to development of T1D. In 2021 approximately 8.4 million individuals were reported to have T1D worldwide. Of these ∼1.5 million were younger than 20 years, 5.4 million were in the age group of 20-59 years, and 1.6 million were aged 60 years or older. Furthermore, there were 0.5 million new cases diagnosed, and about 35,000 undiagnosed individuals died within 12 months of symptomatic onset in 2021. One-fifth of individuals with T1D are living in low-income and lower-middle-income countries. Alarmingly, this group has predicted an increase in prevalent cases to 13.5-17.4 million in low-income and lower-middle-income countries by 2040 (3). The exact pathophysiology of T1D is still not yet understood but is associated with a wide range of genetic and environmental factors, especially viral infection and nutrition, or a combination of both genetics and environment (4,5,6). Among the various risk factors, having a family history of T1D is associated with an increased risk of developing the disease.

Vitamin D and its receptor are well known to play a prominent role in T1D development (6). Vitamin D deficiency (VDD) is a major health issue in various populations, including India, even with abundant sunshine (7,8). The rate of T1D incidence is reported to be steadily increasing and is associated with VDD across global populations (9,10). The ability of vitamin D to prevent T1D could be attributed to its immunoregulatory effect. Indeed, vitamin D plays a dynamic role in the inhibition of macrophage stimulation, antigen-presenting cell maturation, and dendritic cell differentiation, affecting the cytokine paradigm, and reducing the expression of human leukocyte antigen (HLA) class I molecules and Fas, thereby leading to reduced pancreatic β-cell damage (4,11). Although HLA class I and II loci are known to play a major role in T1D development, non-HLA genes such as the genes for proinflammatory (tumor necrosis factor-α, interferon-γ, IL-1, and IL-6) and anti-inflammatory cytokines (IL-10, IL-12, IL-13 and transforming growth factor-β) and vitamin D associated factors (VDR, CYP2R1, CYP27B1, CYP24A1, and DBP) have also been reported as high-risk factors (12,13,14,15).

VDR is a member of the nuclear hormone receptor family that exhibits a functional effect upon binding to vitamin D. Furthermore, the VDR-vitamin D heterodimerizes with retinoid X receptor. This binds to the vitamin D response element located in the promoter region of vitamin D responsive genes leading to the recruitment of co-activators or co-repressors to regulate the transcription of the genes (16). The *VDR* gene is located on chromosome 12q13.11. This gene contains nine exons and spans ∼ 75 kb of genomic DNA. Principally, exon 2-9 encode 427 amino acids containing VDR protein isoforms that consequence to include the DNA-binding (2-3 exon) and the vitamin D-binding (5-9 exon) regions (17). More than 8700 *VDR* gene polymorphisms have been described in healthy and various disease conditions among various populations (https://www.ncbi.nlm.nih.gov/pmc/). Particularly, *VDR* gene single nucleotide polymorphisms (SNPs) at four loci, namely FokI- F/f (rs2228570 C/T), BsmI- B/b (rs1544410 T/C), ApaI- A/a (rs7975232 T/C) and TaqI- T/t (rs731236 T/G) are known to be closely involved in vitamin D metabolism and vitamin D levels, and may thereby act as risk factors for developing T1D (18). Previous studies have reported one or more VDR polymorphisms associated with T1D (19,20,21,22,23,24,25,26). However, other studies have failed to lend support to this association (19,27,28,29,30). Vitamin D status and *VDR* gene polymorphisms are yet to be investigated in T1D in various ethnicities, including South Indians. Hence, the present study set out to explore the significance of vitamin D levels and *VDR* gene polymorphisms and the risk of T1D in the South Indian population.

Methods

Study Subjects

Participants including patients diagnosed with T1D and unaffected relatives [comprising parents, first-degree relatives (FDR), and 36 trios] belonging to 120 families were recruited from Government Rajaji Hospital, Madurai, Tamil Nadu, India. The T1D patients were diagnosed and stratified for inclusion in this study based on the American Diabetes Association guidelines (31). Approximately 5 mL of venous blood was obtained from all participating individuals throughout the year because of the climate in the country.

DNA Extraction and Serum Separation

The serum was separated from ∼1.5 mL of clotted blood. In addition, genomic DNA was extracted from ∼ 3.5 mL of ethylenediaminetetraacetic acid blood by the salting-out method (32). Serum and genomic DNA were stored at - 80 °C and -20 °C, respectively, for further analysis.

Estimation of Serum 25 (OH)-vitamin D Levels

Serum 25-hydroxyvitamin D_2 and D_3 [25 (OH)-D_2 and 25 (OH)-D_3] levels were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (DIA source Immunoassays S.A., Belgium). The serum 25 (OH) vitamin D status was classified as VDD ≤10 ng/dL, vitamin D insufficiency (VDI) 11-30 ng/dL, vitamin D sufficiency (VDS) 31-50 ng/dL and vitamin D toxicity (VDT) > 100 ng/dL, based on the Clinical Practice Guidelines of the Endocrine Society (33).

VDR Genotyping

The VDR FokI-F/f (rs2228570 T > C), BsmI-B/b (rs1544410 A > G), ApaI-A/a (rs7975232 C > A), and TaqI-T/t (rs731236 T > C) polymorphisms were analyzed using amplification refractive mutation system-polymerase chain reaction method, as previously described (34).

This study was approved by the Institutional Ethics Committees of Madurai Kamaraj University (MKU/IRB/11/11) and Government Rajaji Hospital (ref no: 23339/E4/3/10, date: 12.04.2011). The participant's written informed consent was obtained along with a detailed questionnaire.

Statistical Analysis

Vitamin D levels were compared between T1D patients and siblings by Student's t-test. Prior to the familial association analysis, genotype data of 36 trio families were assessed for quality control. Out of 36 families, 33 trios qualified for further analyses. Single-point and multi-point association analyses were carried out for the transmission disequilibrium test (TDT). To obtain empirical p values, 10,000 permutations were run for each analysis, and a p value of < 0.05 was considered statistically significant. TDT and Linkage disequilibrium (LD) analyses were performed using Haploview 4.2v (35).

The association of alleles, genotypes, and haplotypes with T1D was tested through odds ratio (OR) with a 95% confidence interval. Logistic regression analysis was conducted to correlate VDR SNPs with serum vitamin D status. Continuous and categorical variables are presented as mean ± standard deviation and percentage, respectively. All exploratory data analyses were performed using Epi-info 7v (https://www.cdc.gov/epiinfo/index.html), R programme (www.R-project.org/), and STATA 14v (College Station, TX: StataCorp LLC). The level of significance was set at a $p < 0.05$.

Bioinformatics Analysis

Pathogenicity prediction scores of nonsynonymous (ns) VDR-FokI (rs2228570) SNP was performed using SIFT, SIFT4G, Polyphen2_HDIV, Polyphen2_HVAR, LRT, MutationTaster, MetaSVM, MetaLR, PrimateAI, DEOGEN2, BayesDel_addAF, BayesDel_noAF, ClinPred, and LIST-S2 tools, which are genome-scale based. These query variants can be classified as likely pathogenic/deleterious or neutral/benign properties by the above 14 tools through dbNSFP V4.1a on VannoPortal (36,37). This portal also archives five major classes of annotations including basic SNP information, evolutionary annotation, disease association, SNP regulatory potential, and SNP pathogenicity and variant pathogenicity.

Table 1. Demographical and clinical categorization of T1D patients and their family members in the study

	T1D patients and age at onset				First degree relatives	
Characteristics	**All patients**	**< 10 yrs**	**11-20 yrs**	**> 20 yrs**	**Parents**	**Siblings**
No. of patients	120	34	56	30	120	94
Age (year)						
Mean ± SD	24.10 ± 10.07	16.11 ± 6.42	30.31 ± 14.08	30.31 ± 14.21	42.92 ± 9.92	20.55 ± 12.33
Range	4-50	4-32	13-40	23-50	25-72	1.5-65
Gender						
Male	70 (57.78%)	20 (58.82%)	32 (56.90%)	17 (56.67%)	44 (36.67%)	56 (59.57%)
Female	51 (42.14%)	14 (41.18%)	24 (43.10%)	13 (43.33%)	76 (63.33%)	38 (40.42%)
Disease duration						
< 2 yrs	4 (3.28%)	2 (5.88%)	10 (17.24%)	2 (6.67%)	-	-
2-5 yrs	41 (34.43%)	6 (17.65%)	17 (32.76%)	7 (23.33%)	-	-
6-10 yrs	37 (30.33)	13 (38.23%)	16 (27.59%)	8 (26.67%)	-	-
11-15 yrs	19 (15.57%)	5 (14.71%)	7 (12.07%)	7 (23.33%)	-	-
> 15 yrs	20 (16.39%)	8 (23.53%)	6 (10.34%)	6 (20.00%)	-	-

First-degree relatives are comprised of parents and siblings; continuous variables are expressed as mean ± SD; categorical variables are expressed as n (%).
T1D: type 1 diabetes, yrs: years, SD: standard deviation

Results

A total of 334 participants (120 T1D patients and 214 controls) belonging to 120 families (comprising parents, FDR, and 36 trios) were recruited. The detailed demographic and clinical variables of the T1D patients and their FDRs are presented in Table 1. All 334 individuals were characterized by VDR genotyping. Furthermore, serum vitamin D levels were measured in 173 (51.7%), including 98 (81.7%) T1D patients and 75 age- and sex-matched siblings.

Vitamin D Status

Serum vitamin D levels and their status distribution in T1D patients and siblings are presented in Table 2 and Figure 1. Vitamin D [25 (OH)] level was significantly lower in T1D patients (9.73 ± 7.82 ng/dL) than in healthy siblings (16.17 ± 11.48 ng/dL). The distribution of vitamin D status among T1D patients and siblings was: VDD 52% vs 36% respectively, VDI 43.9% vs 53.1%, respectively, and VDS 4.1% vs 13%, respectively. However, no participant was classified as VDT in the study population.

VDR Polymorphisms in Trios

TDT analysis revealed that VDR-ApaI-A (T: U = 15/5; p = 0.006) and VDR-BsmI-B (T: U = 17/5; p = 0.01) alleles were significantly over-transmitted from parents to T1D patients. However, the VDR-FokI-F and VDR-TaqI-T alleles did not show any deviation in allele transmission. Furthermore, haplotype BsmI-b/ApaI-a/TaqI-T (baT) was significantly under-transmitted (T: U = 5.4/14.4; p = 0.04) from parents to T1D patients (Table 3).

LD analysis identified a single haplotype block, comprising VDR TaqI-ApaI-BsmI (Figure 2). High LD was observed between TaqI-ApaI (D' = 1.0; LOD = 14.02; r2 = 0.45), TaqI-BsmI (D' = 0.92; LOD = 19.9; r2 = 0.68) and BsmI-ApaI (D' = 1.0; LOD = 18.73; r2 = 0.62).

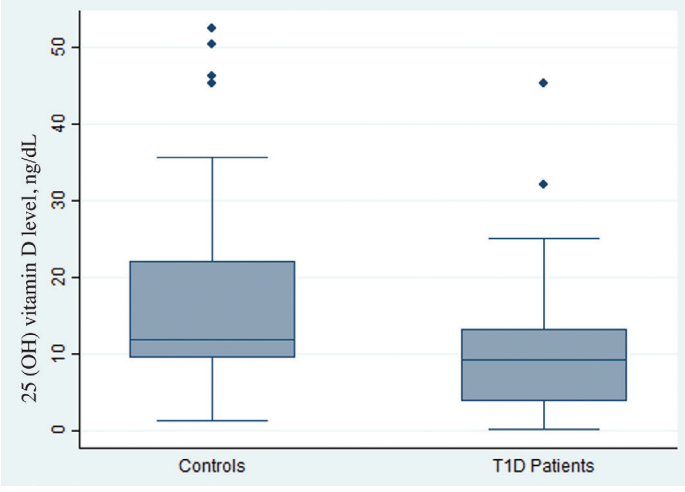

Figure 1. Serum 25 (OH) vitamin D levels (ng/dL) in T1D patients and controls

T1D: type 1 diabetes

VDR Polymorphisms in Patients and FDRs

Allele and genotype frequencies of VDR polymorphisms (FokI, BsmI, ApaI, and TaqI) are presented in Table 4. The genotype frequency of FokI-FF (OR = 1.66; p = 0.03) was significantly more common in T1D patients than FDRs, while the FokI-Ff genotype frequency (OR = 0.5; p = 0.02) was significantly lower in T1D patients than FDRs. However, genotype and allele frequencies of BsmI, ApaI,

Table 2. Vitamin D status and its distribution among T1D patients and siblings

Parameter	T1D patients (%)	Siblings (%)	p
Subjects	98	75	-
Vitamin D, ng/mL	9.73 ± 7.82	16.17 ± 11.48	**0.00001**
Deficiency (%)	51 (52.0)	27 (36.0)	**0.03**
Insufficiency (%)	43 (43.9)	39 (52.0)	0.29
Sufficiency (%)	4 (4.1)	9 (12.0)	0.05

T1D: type 1 diabetes

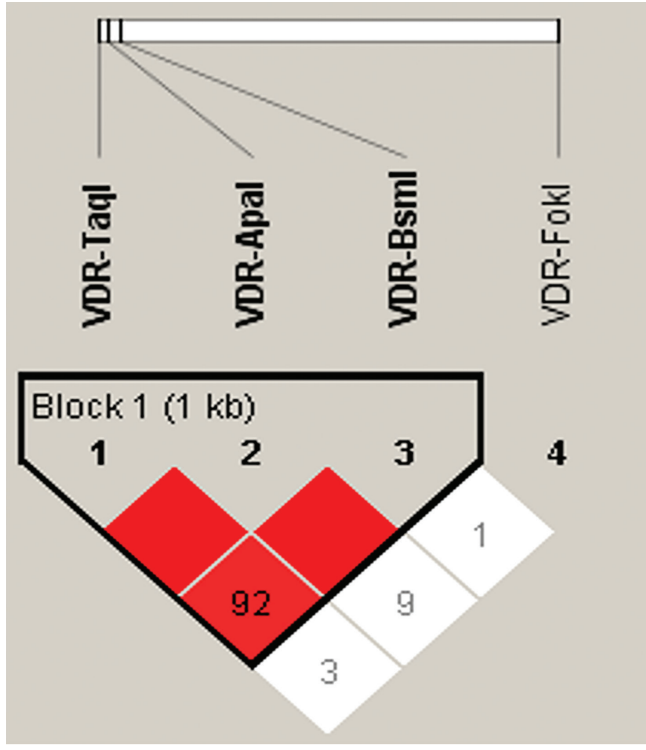

Figure 2. LD plots of the *VDR* gene polymorphism associated with T1D: The physical position of each SNP is shown above the plot as a white bar. The black outline denotes the haplotype block. Color intensity indicates the value of D' from white (D' = 0) to red (D' = 1)

VDR: vitamin D receptor, LD: Linkage disequilibrium, SNP: single nucleotide polymorphism

Table 3. TDT analysis of VDR alleles and haplotypes transmission from parents to siblings in trio families

Genotype/haplotype	T:U	MAF	χ^2 value	p
VDR-Allele				
TaqI-T	18:14	0.23	0.5	0.48
ApaI-A	18:05	0.47	7.3	**0.006**
BsmI-B	17:05	0.38	6.5	**0.01**
FokI-F	12:10	0.41	0.18	0.67
VDR-Haplotype				
BsmI-b/ApaI-A/TaqI-T	14.9:8.8	0.39	1.6	0.2
BsmI-b/ApaI-a/TaqI-T	5.4:14.4	0.38	4.1	**0.04**
BsmI-B/ApaI-A/TaqI-t	7.4:8.6	0.11	0.09	0.76
BsmI-B/ApaI-A/TaqI-T	5.4:1.2	0.07	2.64	0.1
BsmI-b/ApaI-A/TaqI-t	1.1:1.2	0.02	0.91	0.91

T: U copies of the minor allele transmitted (T) and non-transmitted (U) from heterozygous parents to affected offspring; MAF minor allele frequencies in type 1 diabetes (T1D) patients.
VDR: vitamin D receptor, TDT: transmission disequilibrium test

and TaqI did not show significant differences between the study groups. Likewise, there were no significant differences in the distribution of *VDR* genotypes and haplotypes based on the age at onset of the disease (data not shown). Logistic regression analysis of *VDR* genotypes and vitamin D status (VDD, VDI, and VDS) did not show a significant association with T1D (Supplementary Table 1).

Discussion

Vitamin D status is reported to be associated with a predisposition to immunological disorders such as T1D, rheumatoid arthritis, dermatomyositis, multiple sclerosis, systemic lupus erythematosus, hepatitis, asthma, inflammatory bowel disease, and microbial infections (38). Indeed, Vitamin D exerts immune-modulatory

Table 4. Distribution of genotype and allele frequencies of *VDR* gene polymorphisms in T1D patients and controls

VDR-SNPs	Patients, n = 120 (%)	Controls, n = 214 (%)	OR	95% CI	p
FokI-genotype					
FF	76 (63.33)	109 (50.94)	1.6639	1.0526-2.6302	**0.03**
Ff	39 (32.5)	98 (45.79)	0.5699	0.3572-0.9093	**0.02**
ff	5 (4.17)	7 (3.27)	1.2857	0.3990-4.1429	0.67
Allele					
F	191 (79.58)	316 (73.83)	1.3816	0.9441-2.0217	0.09
f	49 (20.42)	112 (26.17)			
BsmI-genotype					
BB	34 (28.33)	76 (35.51)	0.7179	0.4416-1.1669	0.18
Bb	50 (41.67)	81 (37.85)	1.1728	0.7433-1.8506	0.49
bb	36 (30.0)	57 (26.64)	1.1805	0.7201-1.9351	0.51
Allele					
B	118 (49.17)	233 (54.44)	0.8095	0.5898-1.1110	0.19
b	122 (50.83)	195 (45.56)			
ApaI-genotype					
AA	20 (16.67)	34 (15.89)	1.0588	0.5788-1.9371	0.85
Aa	53 (44.16)	102 (47.66)	0.8686	0.5543-1.3611	0.53
aa	47 (39.17)	78 (36.45)	1.1226	0.7085-1.7788	0.62
Allele					
A	93 (38.75)	170 (39.72)	0.9601	0.6945-1.3274	0.8
a	147 (61.25)	158 (60.28)			
TaqI-genotype					
TT	25 (20.83)	35 (16.36)	1.3459	0.7608-2.3807	0.3
Tt	54 (45.00)	102 (47.66)	0.8984	0.5736-1.4071	0.63
tt	41 (34.17)	77 (35.98)	0.9234	0.5775-1.4764	0.73
Allele					
T	104 (43.33)	172 (40.19)	1.1382	0.8263-1.5677	0.42
t	136 (56.67)	256 (59.81)			

OR: odds ratio; 95% CI: 95% confidence interval, T1D: type 1 diabetes, VDR: vitamin D receptor, SNPs: single nucleotide polymorphisms

effects, such as successive reduction in T-cell infiltration, decrease in proinflammatory cytokines, and suppression of the autoimmune process, which could be expected to delay the development of T1D (38). Moreover, vitamin D supplementation for women during pregnancy and infant life has been recognized as a protective factor against T1D (39,40,41). Similarly, vitamin D treatment potentially improves glycemic control in T1D children and adolescents (42). Although the significance of vitamin D status in T1D patients is widely recognized, the influence of VDD on T1D incidence remains unclear (43). The present study findings show that VDD is more common in diagnozed patients with T1D than in their unaffected relatives, which is in agreement with the earlier reports from Swedish, Qatari, North Indian, Iranian, American, Egyptian, Australian, Italian, Saudi Arabian, and Bangladesh populations (44,45,46,47,48,49,50,51,52 ,53). Further, a meta-analysis study also reported that VDD was significantly more common in the T1D group (10). Nevertheless, a lower VDD frequency is reported in Quatrain and Egyptian populations (24,44).

Vitamin D pathway genes, such as *VDR, CYP27A1, CYP27B1, CYP2R1, CYP24A1*, and vitamin D binding protein (*VDBP*) not only control vitamin D biosynthesis and its transport but also influence the cytokine levels in various autoimmune diseases, including T1D (54,55,56). Vitamin D plays a major role in the regulation of insulin secretion from pancreatic β-cells (57,58). VDR polymorphisms in the 3´UTR region are well known to affect its translation and mRNA stability. Predominantly, the BsmI, ApaI, TaqI, and FokI SNPs are the most commonly studied *VDR* gene polymorphisms in association with non-skeletal outcomes, including T1D (59). However, limited familial studies are reporting the transmission of VDR alleles, genotypes, and haplotypes from parents to T1D-affected children (27,30,60,61,62,63,64,65,66).

In the present study, the BsmI-B and ApaI-A alleles were over-transmitted, apparently conferring susceptibility and BsmI-b/ApaI-a/TaqI-T (baT) haplotype was under-transmitted, suggesting protection to T1D. Further, the study also revealed a strong LD between TaqI-ApaI, TaqI-BsmI, and BsmI-ApaI. This is in accord with previous reports on VDR TaqI-ApaI in German, British and Egyptian populations (60,61,67). The VDR FokI polymorphism affects immune cell behavior and possibly plays a role in immune-mediated diseases, including T1D (68). In the present study, FokI-FF and FokI-Ff genotypes were significantly associated with susceptibility and protection to T1D, respectively. The T1D susceptibility association with the FokI-FF genotype has been previously reported in various populations (19,20,21,22,23,24,25,26). However, this is not consistently found. Several studies have shown a lack of association of the VDR-Folk-FF genotype with T1D susceptibility (28,68,69,70,71,72,73,74). The alleles and haplotypes of VDR FokI, BsmI, ApaI, and TaqI and genotypes of BsmI, ApaI, and TaqI were not associated with T1D in the current study. Similarly, the BsmI/ApaI/TaqI alleles and genotypes have not shown an association with T1D in several other populations (21,22,75,76,77). Furthermore, VDR haplotypes did not show an association with T1D in Spanish, Portuguese, North Indian, and Turkish populations (22,28,69,71). However, several studies point out that VDR BsmI/ApaI/TaqI polymorphisms are associated with T1D (21,24,25,29,70,72,75,78). In addition, meta-analysis studies in Asian populations revealed an association between VDR-BsmI polymorphism and T1D (79,80,81).

The VDR-FokI (rs2228570) SNP leads to alteration in the amino acid sequence that may further affect protein function. This T → C substitution changes the first start codon of ATG by an alternative start site codon of ACG leading to a different-sized protein, such as short-form (424 aa; C allele or F allele, methionine at the 4th position) and long-form (427 aa; T allele or f allele, methionine at 1st position). The 424 aa (mutant) containing VDR is somewhat more active than the 427 aa (wild type) VDR (82,83). Moreover, this transition is predicted to enhance VDR protein stability (84). In this study, VDR-FokI (rs2228570) was slightly associated with T1D in terms of higher f → F allele transition in patients than controls. Although the VDR protein was more stable in T1D patients, they might be susceptible to disease because of the low Vitamin D levels in the patients.

Study Limitations

As this study was structured for a family-based approach, the small sample size is the foremost limitation. A comprehensive familial study with large sample size, DNA sequencing, and gene expression evaluations are necessary to clarify the role of the *VDR* gene variants on T1D in the future. Furthermore, factors possibly influencing serum vitamin D synthesis, such as intake of supplements, obesity, liver and kidney diseases, and cutaneous factors, were not investigated.

Conclusion

The present study found that VDD was more common in patients with T1D than their unaffected relatives in the South Indian population. Furthermore, the VDR polymorphisms FokI-FF genotype, BsmI-B, and ApaI-A alleles were positively associated with T1D. However, the

FokI-Ff genotype and BsmI-b/ApaI-a/TaqI-T haplotype were negatively associated with the disease. Although VDR protein stability is enhanced in subjects harboring the F allele, and was more common in the patients with T1D, this suggests some other Vitamin D associated mechanism or function may be associated with the development of T1D in this population, rather than the simple presence of the FokI-Ff genotype, which was also present in more than 50 % of unaffected related controls.

Ethics

Ethical Committee Approval: This study was approved by the Institutional Ethics Committees of Madurai Kamaraj University (MKU/IRB/11/11) and Government Rajaji Hospital (ref no: 23339/E4/3/10, date: 12.04.2011).

Informed Consent: The participant's written informed consent was obtained along with a detailed questionnaire.

Authorship Contributions

Surgical and Medical Practices: Ayyappan Chitra, Arthur Asirvatham, Concept: Ramasamy Thirunavukkarasu, Ayyappan Chitra, Arthur Asirvatham, Mariakuttikan Jayalakshmi, Design: Ramasamy Thirunavukkarasu, Ayyappan Chitra, Arthur Asirvatham, Mariakuttikan Jayalakshmi, Data Collection or Processing: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi, Analysis or Interpretation: Ramasamy Thirunavukkarasu, Literature Search: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi, Writing: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi.

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References

1. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nat Rev Endocrinol 2009;5:219-226.
2. Tsang VHM, McGrath RT, Clifton-Bligh RJ, Scolyer RA, Jakrot V, Guminski AD, Long GV, Menzies AM. Checkpoint Inhibitor-Associated Autoimmune Diabetes Is Distinct From Type 1 Diabetes. J Clin Endocrinol Metab 2019;104:5499-5506.
3. Gregory GA, Robinson TIG, Linklater SE, Wang F, Colagiuri S, de Beaufort C, Donaghue KC; International Diabetes Federation Diabetes Atlas Type 1 Diabetes in Adults Special Interest Group; Magliano DJ, Maniam J, Orchard TJ, Rai P, Ogle GD. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. Lancet Diabetes Endocrinol 2022;10:741-760. Epub 2022 Sep 13
4. Van Belle TL, Gysemans C, Mathieu C. Vitamin D and diabetes: the odd couple. Trends Endocrinol Metab 2013;24:561-568. Epub 2013 Aug 21
5. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet 2016;387:2331-2339.
6. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet 2016;387:2340-2348.
7. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, Pierroz DD, Weber P, Hoffmann K. A systematic review of vitamin D status in populations worldwide. Br J Nutr 2014;111:23-45. Epub 2013 Aug 9
8. Kumar GT, Chugh R, Eggersdorfer M. Poor Vitamin D Status in Healthy Population in India: A Review of Current Evidence. Int J Vitam Nutr Res. 2015;85:185-201. Epub 2015 Nov 17
9. Mohr SB, Garland CF, Gorham ED, Garland FC. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. Diabetologia 2008;51:1391-1398. Epub 2008 Jun 12
10. De Oliveira VR, Domingueti CP. Association of vitamin D deficiency and type 1 diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract. 2018:280-288.
11. Riachy R, Vandewalle B, Moerman E, Belaich S, Lukowiak B, Gmyr V, Muharram G, Kerr Conte J, Pattou F. 1,25-Dihydroxyvitamin D3 protects human pancreatic islets against cytokine-induced apoptosis via down-regulation of the Fas receptor. Apoptosis 2006;11:151-159.
12. Cooper JD, Smyth DJ, Walker NM, Stevens H, Burren OS, Wallace C, Greissl C, Ramos-Lopez E, Hyppönen E, Dunger DB, Spector TD, Ouwehand WH, Wang TJ, Badenhoop K, Todd JA. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. Diabetes 2011;60:1624-1631. Epub 2011 Mar 25
13. Wållberg M, Cooke A. Immune mechanisms in type 1 diabetes. Trends Immunol 2013;34:583-591. Epub 2013 Sep 18
14. Noble JA. Immunogenetics of type 1 diabetes: A comprehensive review. J Autoimmun 2015;64:101-112. Epub 2015 Aug 10
15. Penna-Martinez M, Badenhoop K. Inherited Variation in Vitamin D Genes and Type 1 Diabetes Predisposition. Genes (Basel) 2017;8:125.
16. Meyer MB, Pike JW. Corepressors (NCoR and SMRT) as well as coactivators are recruited to positively regulated 1α,25-dihydroxyvitamin D3-responsive genes. J Steroid Biochem Mol Biol 2013;136:120-124. Epub 2012 Aug 25
17. Zenata O, Vrzal R. Fine tuning of vitamin D receptor (VDR) activity by post-transcriptional and post-translational modifications. Oncotarget 2017;8:35390-35402.
18. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2011;376:180-188. Epub 2010 Jun 10
19. Ban Y, Taniyama M, Yanagawa T, Yamada S, Maruyama T, Kasuga A, Ban Y. Vitamin D receptor initiation codon polymorphism influences genetic susceptibility to type 1 diabetes mellitus in the Japanese population. BMC Med Genet 2001;2:7. Epub 2001 Jun 25

20. Zemunik T, Skrabic V, Boraska V, Diklic D, Terzic IM, Capkun V, Peruzovic M, Terzic J. FokI polymorphism, vitamin D receptor, and interleukin-1 receptor haplotypes are associated with type 1 diabetes in the Dalmatian population. J Mol Diagn 2005;7:600-604.
21. Panierakis C, Goulielmos G, Mamoulakis D, Petraki E, Papavasiliou E, Galanakis E. Vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Crete, Greece. Clin Immunol 2009;133:276-281. Epub 2009 Sep 5
22. Israni N, Goswami R, Kumar A, Rani R. Interaction of vitamin D receptor with HLA DRB1 0301 in type 1 diabetes patients from North India. PLoS One 2009;4:e8023.
23. Kocabaş A, Karagüzel G, Imir N, Yavuzer U, Akçurin S. Effects of vitamin D receptor gene polymorphisms on susceptibility to disease and bone mineral density in Turkish patients with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2010;23:1289-1297.
24. Abd-Allah SH, Pasha HF, Hagrass HA, Alghobashy AA. Vitamin D status and vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Egyptian children. Gene 2014;536:430-434. Epub 2013 Dec 23
25. Mukhtar M, Batool A, Wajid A, Qayyum I. Vitamin D Receptor Gene Polymorphisms Influence T1D Susceptibility among Pakistanis. Int J Genomics 2017;2017:4171254. Epub 2017 Dec 3
26. Ali R, Fawzy I, Mohsen I, Settin A. Evaluation of vitamin D receptor gene polymorphisms (Fok-I and Bsm-I) in T1DM Saudi children. J Clin Lab Anal 2018;32:e22397. Epub 2018 Feb 8
27. McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, Snehalatha C, Hitman GA. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 1997;8:971-975.
28. Lemos MC, Fagulha A, Coutinho E, Gomes L, Bastos M, Barros L, Carrilho F, Geraldes E, Regateiro FJ, Carvalheiro M. Lack of association of vitamin D receptor gene polymorphisms with susceptibility to type 1 diabetes mellitus in the Portuguese population. Hum Immunol 2008;69:134-138.
29. Motohashi Y, Yamada S, Yanagawa T, Maruyama T, Suzuki R, Niino M, Fukazawa T, Kasuga A, Hirose H, Matsubara K, Shimada A, Saruta T. Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. J Clin Endocrinol Metab 2003;88:3137-3140.
30. Turpeinen H, Hermann R, Vaara S, Laine AP, Simell O, Knip M, Veijola R, Ilonen J. Vitamin D receptor polymorphisms: no association with type 1 diabetes in the Finnish population. Eur J Endocrinol 2003;149:591-596.
31. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care 2018;41(Suppl 1):13-27.
32. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res1988;16:1215.
33. 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
34. Jafari M, Pirouzi A, Anoosheh S, Farnia P, Tajik N. Rapid and simultaneous detection of vitamin D receptor gene polymorphisms by a single ARMS-PCR assay. Mol Diagn Ther 2014;18:97-103.
35. Liu X, Li C, Mou C, Dong Y, Tu Y. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med 2020;12:103.
36. Huang D, Zhou Y, Yi X, Fan X, Wang J, Yao H, Sham PC, Hao J, Chen K, Li MJ. VannoPortal: multiscale functional annotation of human genetic variants for interrogating molecular mechanism of traits and diseases. Nucleic Acids Res 2022;50:1408-1416.
37. Marino R, Misra M. Extra-Skeletal Effects of Vitamin D. Nutrients 2019;11:1460.
38. Infante M, Ricordi C, Sanchez J, Clare-Salzler MJ, Padilla N, Fuenmayor V, Chavez C, Alvarez A, Baidal D, Alejandro R, Caprio M, Fabbri A. Influence of Vitamin D on Islet Autoimmunity and Beta-Cell Function in Type 1 Diabetes. Nutrients 2019;11:2185.
39. Marjamäki L, Niinistö S, Kenward MG, Uusitalo L, Uusitalo U, Ovaskainen ML, Kronberg-Kippilä C, Simell O, Veijola R, Ilonen J, Knip M, Virtanen SM. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia 2010;53:1599-1607. Epub 2010 Apr 6
40. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes 2012;61:175-178. Epub 2011 Nov 28
41. Giri D, Pintus D, Burnside G, Ghatak A, Mehta F, Paul P, Senniappan S. Treating vitamin D deficiency in children with type I diabetes could improve their glycaemic control. BMC Res Notes 2017;10:465.
42. Rak K, Bronkowska M. Immunomodulatory Effect of Vitamin D and Its Potential Role in the Prevention and Treatment of Type 1 Diabetes Mellitus-A Narrative Review. Molecules 2018;24:53.
43. Littorin B, Blom P, Schölin A, Arnqvist HJ, Blohmé G, Bolinder J, Ekbom-Schnell A, Eriksson JW, Gudbjörnsdottir S, Nyström L, Ostman J, Sundkvist G. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006;49:2847-2852. Epub 2006 Oct 27
44. Bener A, Alsaied A, Al-Ali M, Al-Kubaisi A, Basha B, Abraham A, Guiter G, Mian M. High prevalence of vitamin D deficiency in type 1 diabetes mellitus and healthy children. Acta Diabetol 2009;46:183-189. Epub 2008 Oct 10
45. Borkar VV, Devidayal, Verma S, Bhalla AK. Low levels of vitamin D in North Indian children with newly diagnosed type 1 diabetes. Pediatr Diabetes 2010;11:345-350.
46. Ghandchi Z, Neyestani TR, Yaraghi AA, Eshraghian MR, Gharavi A, Shariatzadeh N, Kalayi A, Houshiarrad A. Vitamin D status and the predictors of circulating T helper 1-type immunoglobulin levels in Iranian subjects with type 1 diabetes and their siblings: a case-control study. J Hum Nutr Diet 2012;25:365-372. Epub 2012 Feb 17
47. Gorham ED, Garland CF, Burgi AA, Mohr SB, Zeng K, Hofflich H, Kim JJ, Ricordi C. Lower prediagnostic serum 25-hydroxyvitamin D concentration is associated with higher risk of insulin-requiring diabetes: a nested case-control study. Diabetologia 2012;55:3224-3227. Epub 2012 Sep 7
48. Azab SF, Saleh SH, Elsaeed WF, Abdelsalam SM, Ali AA, Esh AM. Vitamin D status in diabetic Egyptian children and adolescents: a case-control study. Ital J Pediatr 2013;39:73.
49. Greer RM, Portelli SL, Hung BS, Cleghorn GJ, McMahon SK, Batch JA, Conwell LS. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes 2013;14:31-41. Epub 2012 Aug 23
50. Franchi B, Piazza M, Sandri M, Mazzei F, Maffeis C, Boner AL. Vitamin D at the onset of type 1 diabetes in Italian children. Eur J Pediatr 2014;173:477-482. Epub 2013 Nov 13

51. Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Yakout SM, Aljohani NJ, Al Fawaz H, Al-Ajlan AS, Sheshah ES, Al-Yousef M, Alharbi M. Lower vitamin D status is more common among Saudi adults with diabetes mellitus type 1 than in non-diabetics. BMC Public Health 2014;14:153.

52. Zabeen B, Nahar J, Ahmed B, Tayyeb S, Islam N, Azad K. Vitamin D status in children and adolescents with type 1 diabetes in a specialized diabetes care centre in Bangladesh. Endocrinol Diabetes Metab 2022;5:e00312. Epub 2021 Nov 12

53. Yang CY, Leung PS, Adamopoulos IE, Gershwin ME. The implication of vitamin D and autoimmunity: a comprehensive review. Clin Rev Allergy Immunol 2013;45:217-226.

54. Tangjittipokin W, Umjai P, Khemaprasit K, Charoentawornpanich P, Chanprasert C, Teerawattanapong N, Narkdontri T, Santiprabhob J. Vitamin D pathway gene polymorphisms, vitamin D level, and cytokines in children with type 1 diabetes. Gene 2021;791:145691. Epub 2021 May 5

55. Khammissa RAG, Fourie J, Motswaledi MH, Ballyram R, Lemmer J, Feller L. The Biological Activities of Vitamin D and Its Receptor in Relation to Calcium and Bone Homeostasis, Cancer, Immune and Cardiovascular Systems, Skin Biology, and Oral Health. Biomed Res Int 2018;2018:9276380.

56. Miettinen ME, Smart MC, Kinnunen L, Harjutsalo V, Reinert-Hartwall L, Ylivinkka I, Surcel HM, Lamberg-Allardt C, Hitman GA, Tuomilehto J. Genetic determinants of serum 25-hydroxyvitamin D concentration during pregnancy and type 1 diabetes in the child. PLoS One 2017;12:e0184942.

57. Kirac D, Dincer Yazan C, Gezmis H, Yaman A, Haklar G, Sirikci O, Altunok EC, Deyneli O. VDBP, VDR Mutations and Other Factors Related With Vitamin D Metabolism May Be Associated With Type 1 Diabetes Mellitus. Cell Mol Biol (Noisy-le-grand) 2018;64:11-16.

58. Ruiz-Ojeda FJ, Anguita-Ruiz A, Leis R, Aguilera CM. Genetic Factors and Molecular Mechanisms of Vitamin D and Obesity Relationship. Ann Nutr Metab 2018;73:89-99. Epub 2018 Jul 6

59. Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, Badenhoop K. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. Diabetes 2000;49:504-507.

60. Nejentsev S, Cooper JD, Godfrey L, Howson JM, Rance H, Nutland S, Walker NM, Guja C, Ionescu-Tirgovişte C, Savage DA, Undlien DE, Rønningen KS, Tuomilehto-Wolf E, Tuomilehto J, Gillespie KM, Ring SM, Strachan DP, Widmer B, Dunger D, Todd JA. Analysis of the vitamin D receptor gene sequence variants in type 1 diabetes. Diabetes 2004;53:2709-2712.

61. Angel B, Santos JL, Carrasco E, Albala C, Pérez-Bravo F. Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in Chilean subjects: a case-parent study. Eur J Epidemiol 2004;19:1085-1087.

62. Ramos-Lopez E, Jansen T, Ivaskevicius V, Kahles H, Klepzig C, Oldenburg J, Badenhoop K. Protection from type 1 diabetes by vitamin D receptor haplotypes. Ann N Y Acad Sci 2006;1079:327-334.

63. Mimbacas A, Trujillo J, Gascue C, Javiel G, Cardoso H. Prevalence of vitamin D receptor gene polymorphism in a Uruguayan population and its relation to type 1 diabetes mellitus. Genet Mol Res 2007;6:534-542.

64. Boraska V, Škrabić V, Zeggini E, Groves CJ, Buljubašić M, Peruzović M, Zemunik T. Family-based analysis of vitamin D receptor gene polymorphisms and type 1 diabetes in the population of South Croatia. J Hum Genet 2008;53:210-214. Epub 2007 Dec 27

65. Thorsen SU, Mortensen HB, Carstensen B, Fenger M, Thuesen BH, Husemoen L, Bergholdt R, Brorsson C, Pociot F, Linneberg A, Svensson J. No association between type 1 diabetes and genetic variation in vitamin D metabolism genes: a Danish study. Pediatr Diabetes 2013;15:416-421. Epub 2013 Dec 10

66. Elgazzaz MG, Mohammed EAE, Atwa HA, Badr FM. Association of Vitamin D Receptor Gene Polymorphisms and Type1 Diabetes in an Egyptian Population. Mol Biol 2016;5:3-6.

67. Audí L, Martí G, Esteban C, Oyarzabal M, Chueca M, Gussinyé M, Yeste D, Fernández-Cancio M, Andaluz P, Carrascosa A. VDR gene polymorphism at exon 2 start codon (FokI) may have influenced Type 1 diabetes mellitus susceptibility in two Spanish populations. Diabet Med 2004;21:393-394.

68. San-Pedro JI, Bilbao JR, Perez de Nanclares G, Vitoria JC, Martul P, Castaño L. Heterogeneity of vitamin D receptor gene association with celiac disease and type 1 diabetes mellitus. Autoimmunity 2005;38:439-444.

69. Mory DB, Rocco ER, Miranda WL, Kasamatsu T, Crispim F, Dib SA. Prevalence of vitamin D receptor gene polymorphisms FokI and BsmI in Brazilian individuals with type 1 diabetes and their relation to beta-cell autoimmunity and to remaining beta-cell function. Hum Immunol 2009;70:447-451. Epub 2009 Mar 12

70. Gogas Yavuz D, Keskin L, Kıyıcı S, Sert M, Yazıcı D, Şahin İ, Yüksel M, Deyneli O, Aydın H, Tuncel E, Akalın S. Vitamin D receptor gene BsmI, FokI, ApaI, TaqI polymorphisms and bone mineral density in a group of Turkish type 1 diabetic patients. Acta Diabetol 2011;48:329-336. Epub 2011 May 3

71. Mohammadnejad Z, Ghanbari M, Ganjali R, Afshari JT, Heydarpour M, Taghavi SM, Fatemi S, Rafatpanah H. Association between vitamin D receptor gene polymorphisms and type 1 diabetes mellitus in Iranian population. Mol Biol Rep 2012;39:831-837. Epub 2011 May 17

72. Hamed EO, Abdel-Aal AM, Din AK, Atia MM. Vitamin D level and Fok-I vitamin D receptor gene polymorphism in Egyptian patients with type-1 diabetes. Egypt J Immunol 2013;20:1-10.

73. Iyer A, Lanham-New S, Khoja S, Al-Ghamdi M, Aldoghaither H. Relationship Between Vitamin D Receptor Gene Polymorphisms and Type 1 Diabetes Mellitus in Saudi Patients. Int J Pharmacol 2017;13:1092-1097.

74. Chang TJ, Lei HH, Yeh JI, Chiu KC, Lee KC, Chen MC, Tai TY, Chuang LM. Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin Endocrinol (Oxf) 2000;52:575-580.

75. Skrabić V, Zemunik T, Situm M, Terzić J. Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in the Dalmatian population. Diabetes Res Clin Pract 2003;59:31-35.

76. García D, Angel B, Carrasco E, Albala C, Santos JL, Pérez-Bravo F. VDR polymorphisms influence the immune response in type 1 diabetic children from Santiago, Chile. Diabetes Res Clin Pract 2007;77:134-140. Epub 2006 Nov 28

77. Shimada A, Kanazawa Y, Motohashi Y, Yamada S, Maruyama T, Ikegami H, Awata T, Kawasaki E, Kobayashi T, Nakanishi K, Kawabata Y, Kurihara S, Uga M, Tanaka S; Japanese Study Group on Type 1 Diabetes Genetics. Evidence for association between vitamin D receptor BsmI polymorphism and type 1 diabetes in Japanese. J Autoimmun 2008;30:207-211. Epub 2007 Oct 23

78. Zhang J, Li W, Liu J, Wu W, Ouyang H, Zhang Q, Wang Y, Liu L, Yang R, Liu X, Meng Q, Lu J. Polymorphisms in the vitamin D receptor gene and type 1 diabetes mellitus risk: an update by meta-analysis. Mol Cell Endocrinol 2012;355:135-142. Epub 2012 Feb 14

79. Wang G, Zhang Q, Xu N, Xu K, Wang J, He W, Yang T. Associations between two polymorphisms (FokI and BsmI) of vitamin D receptor gene and type 1 diabetes mellitus in Asian population: a meta-analysis. PLoS One 2014;9:e89325.

80. Sahin OA, Goksen D, Ozpinar A, Serdar M, Onay H. Association of vitamin D receptor polymorphisms and type 1 diabetes susceptibility

in children: a meta-analysis. Endocr Connect 2017;6:159-171. Epub 2017 Feb 23

81. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 1997;12:915-921.
82. Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H, Tavakkoli P, Galligan MA, Dang HT, Haussler CA, Haussler MR. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 2000;14:401-420.
83. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143-156.
84. Barrett JC, Fry B, Maller JD, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-265.

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Childhood Obesity as a Global Problem: a Cross-sectional Survey on Global Awareness and National Program Implementation

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What is already known on this topic?

The rising trend of childhood obesity worldwide, with its effect on both childhood and adult health, highlights its importance as an international priority. As a multifactorial health challenge, its prevention and management rely on many factors, including establishing a healthy food environment with the involvement and engagement of multiple stakeholders.

What this study adds?

Most countries of the respondents have data and programs targeted at tackling childhood obesity. Levels of support from governments, schools and private organizations as well as awareness regarding World Health Organization and UNICEF guidance vary with progress. Furthermore, implementation was still hindered in most countries.

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Abstract

Objective: The rising global epidemic of childhood obesity is a major public health challenge. Despite the urgency, there is a lack of data on the awareness and implementation of preventative measures. The aim of this study was to identify areas for improvement in the prevention and management of childhood obesity worldwide.

Methods: A cross-sectional electronic survey was distributed to 132 members of national pediatric societies of the International Pediatric Association.

Results: Twenty-eight (21.2%) participants, each from a different country across six World Health Organization (WHO) regions completed the survey. Most participants reported that national prevalence data of childhood obesity is available (78.6%), and the number increased during the Coronavirus disease-2019 pandemic (60.7%). In most countries (78.6%), the amount of sugar and salt in children's products is provided but only 42.9% enacted regulations on children-targeted advertising. Childhood obesity prevention programs from the government (64.3%) and schools (53.6%) are available with existing support from private or non-profit organizations (71.4%). Participants were aware of WHO's guidance concerning childhood obesity (78.6%), while fewer were aware of The United Nations International Children's Emergency Fund's (UNICEF) guidance (50%). Participants reported that WHO/UNICEF guidance acted as a reference to develop policies, regulations and national programs. However, progress was hindered by poor compliance. Lastly, participants provided suggestions on tackling obesity, with responses ranging from developing and reinforcing policies, involvement of schools, and prevention across all life stages.

Conclusion: There are different practices in implementing prevention measures to counter childhood obesity globally, particularly in statutory regulation on food advertising and national programs. While support and awareness was relatively high, implementation was hindered. This reflects the need for prompt, country-specific evaluation and interventions.

Keywords: Childhood obesity, obesity, children, awareness, program

Introduction

The rising epidemic of childhood obesity worldwide is a major public health challenge in the 21st century. Obesity is both a non-communicable disease (NCD) and a modifiable risk factor for many other NCDs, such as cardiovascular diseases, certain types of cancers and type 2 diabetes (1). With less than a decade to the 2030 Sustainable Development Goals (SDGs), the success of achieving these targets is at risk if childhood obesity is not made a global priority. The World Health Organization (WHO) reports that the prevalence of childhood obesity has nearly tripled since 1975, with an estimated 39 million children below the age of 5 years classified as overweight or obese in 2020 and over 340 million children and adolescents between the ages of 5-19 years were overweight or obese in 2016 (2).

With a significant shift in global priorities and disruption to daily routines brought by the Coronavirus disease-2019 (COVID-19) pandemic, studies found that weight gain amongst children and adolescents was on the rise (3). Reduced opportunities for physical activity and proper nutrition due to school closures, increased stress and disrupted routines promoted unhealthy eating habits. Today, children's diets are far from optimal with overconsumption of sugar, sodium and fat, and an insufficient consumption of fruit and vegetables (4). Although the success of the prevention and management of childhood obesity is reliant on many factors, the role of the food environment, defined as the physical, socio-cultural, economic, and political context by which consumers interact with food systems to consume foods (5), also play a significant role.

Products aimed at children constitute a great proportion of the food industry's market segment. Globally, it is estimated that the child-oriented food and beverages market will reach 146.7 billion US dollars by 2025, growing at an annual rate of 5% (6). Food advertising, packaging and regulations controlling the amount of sugar and salt in food products are all important factors influencing children's diets. However, very little is known about the regulations on advertising, packaging, and nutritional components of food products aimed at children globally.

As childhood obesity is a condition of multifactorial origin, successful preventative and management strategies depend on collaboration between multiple stakeholders. The WHO has established several programs, systems, and recommendations for member states on tackling childhood obesity, promoting population-wide policies and initiatives and community-based interventions. With federal and local government jurisdiction over many components of the food environment, the WHO has also recommended key policy actions to restrict promotional marketing, add taxes or levies on sugar-sweetened beverages, and provide regulations for nutrition labeling. The United Nations International Children's Emergency Fund (UNICEF) has also published a step-by-step framework to guide country-level interventions on childhood obesity.

To aid in moving towards the goal of identifying areas for improvement in the prevention and management of childhood obesity, this study reports the global picture of childhood obesity with a focus on the awareness and

implementation of preventative measures for obesity in children, based on an international cross-sectional survey conducted by the International Pediatric Association (IPA).

Methods

Study Setting and Design

An electronic survey was emailed to 132 national pediatric societies across regions under the umbrella of IPA between the 7th and 23rd of January 2022. Google Forms, a web-based survey software automatically collected and stored responses in an electronic spreadsheet. Subjects included in this study were national pediatric societies' presidents or other members representing their respective societies. From each country, only one response was analyzed. To summarize the demographics of the participants and their responses, descriptive statistics were used. Responses to open-ended questions were summarized by approximating the similarity in semantic content. Ethical clearance was not required for this study.

The Survey

Two pediatric endocrinologists, the co-chairs of the IPA Strategic Advisory Group on NCDs, a group of pediatricians specializing in NCDs from different countries across regions, developed the survey questions. The questionnaire was divided into three main sections. The objectives of the survey were explained in the first section. If participants gave consent to participate, they proceed to the next section. The second section included four questions on the demographic details of participants, such as the name of the country, region, name of the pediatric society, and the designation of participants within their respective societies. The third section included 15 questions on the availability of national data on obesity in children and food labels mentioning the amount of sugar and salt, the country's regulation on the advertisement of children's food, programs from the government and schools to prevent obesity, support from private and civil society organizations, awareness of WHO and UNICEF's guidance on childhood obesity, and suggestions to overcome obesity in children. There were multiple-choice and open-ended questions. The survey took approximately 5-10 minutes to complete.

Statistical Analysis

Data obtained in this study was analyzed using two programs, Google Sheets and Statistical Package for the Social Sciences for Windows, version 25.0 (IBM Corp, Armonk, NY' USA). Descriptive statistics were used to describe demographic data and responses to multiple-choice questions. Responses to open-ended questions were analyzed by approximating into similar semantic content. Several questions had results less than the total number of respondents, as they were follow-up questions for certain responses. The final question had more responses than the total number of respondents as respondents were allowed to give multiple answers.

Results

Demographics of Participants

We received 33 responses, with double responses from different participants, each from four different countries (United States, Turkey, Mexico, and Jordan). Based on the data completeness, only one response was included for these four countries, while the other was excluded. Another incomplete response was also excluded. A total of 28 responses from 28 countries across all six WHO regions were included as an illustrative sample to be analyzed.

Participants in this study were predominantly from the European region (35.7%) and the region of the Americas (21.4%). The majority of the participants were from upper-middle-income countries (46.4%) and high-income countries (35.7%). There was no representative from the low-income category, and one participant's country of origin was not listed on the World Bank Classification. Participants were presidents of the pediatric societies and representatives appointed on behalf of the presidents, each contributed 28.6%. The remainder were pediatricians or pediatric endocrinologists who were members of advisory councils in their respective countries (Table 1).

National Program and Regulations Related to Obesity in Children

The national prevalence data about obesity in children was reported to be available by the majority of participants (78.6%). During the COVID-19 pandemic, most participants (60.7%) confirmed an increase in the number of children with obesity, while the rest (39.3%) reported not being aware of the rise in the number.

Information about the amount of sugar and salt in food products for children was provided on the nutrition information label in about four-fifths of participants' countries. However, there was no information available on the maximum amount of sugar and salt reported by most participants (60.7%), as only seven answered "yes" and the remaining four responded "don't know". In addition, advertising rules for children's food packaging are only available in 12 participant countries, while 13 other participants reported such rules were not available.

We asked whether there were any prevention programs or interventions from the government and schools. More than half of the participants confirmed that programs or interventions from the government (64.3%) and school (53.6%) were available (Table 2).

Non-governmental Programs Related to Obesity in Children

Support from private organizations or non-profit organizations (civil society organizations) for childhood obesity prevention programs was reported to be available by most participants (71.4%). Most of these programs were locally and nationally operated (75%) (Table 3).

About two-thirds of participants were aware of regional programs established to prevent childhood obesity (57.1%). However, more than three-quarters of participants were aware of WHO's guidance concerning childhood obesity (78.6%). For those who answered that they were aware of this guidance, they reported that the guidance was used as a reference to develop national policies and regulations (35%) and applied through national programs (15%). Other responses included emphasizing childhood obesity as a priority (5%), increasing awareness of it (5%), participating in the WHO Europe Childhood Obesity Surveillance Initiative (5%) and developing a center for the prevention and treatment of obesity in children and adolescents (5%). However, several participants also reported that programs and regulations existed but progress was hindered by poor compliance (15%), while others were unaware of how the guidance influenced their country's national programs (15%).

In contrast, fewer participants were aware of UNICEF's guidance concerning childhood obesity, with the results being split evenly between those aware and those who were not (50%). Participants responded that UNICEF's guidance had acted as a reference to develop national policies and regulations (28.6%) and applied through national programs (21.4%). Other responses included campaign and advocacy work (14.3%) and increasing the awareness of childhood obesity (14.3%). One participant shared that it had

Table 1. Characteristics of respondent

Characteristics	Number of responses (%)
Region (based on WHO regions)	
South-East Asia Region India, Thailand	2 (7.1)
Western Pacific Region Australia, China, Malaysia, Singapore	4 (14.3)
Eastern Mediterranean Region Jordan, Pakistan, Palestine	3 (10.7)
European Region Bosnia and Herzegovina, Croatia, Ireland, Latvia, Luxembourg, Russian Federation, Serbia, Spain, Turkey, United Kingdom	10 (35.7)
African Region Botswana, Senegal, South Africa	3 (10.7)
Region of the Americas Argentina, Canada, Dominican Republic, Honduras, Mexico, United States	6 (21.4)
Respondent's Country of Origin, based on Income Level (World Bank Classification 2023)	
Low Income	0 (0)
Lower-Middle Income	4 (14.3)
Upper-Middle Income	13 (46.4)
High Income	10 (35.7)
Not classified/No data	1 (3.6)
Designation within the Pediatric Society	
President	8 (28.6)
On behalf of the President	8 (28.6)
Others (advisory group member, member)	12 (42.8)

WHO: World Health Organization

Table 2. National program and regulation related to obesity in children

Characteristics (number)	Number of responses (%)
Availability of national prevalence data of obesity in children (28)	
Yes	22 (78.6)
No	5 (17.9)
Don't know	1 (3.6)
The number of children with obesity increased during the COVID-19 pandemic in your country (28)	
Yes	17 (60.7)
No	0 (0)
Don't know	11 (39.3)
Availability of label on the amount of sugar and salt in food products for children in your country (28)	
Yes	22 (78.6)
No	5 (17.9)
Don't know	1 (3.6)
Availability of a maximum amount of sugar and salt in food products for children in your country's regulation (28)	
Yes	7 (25.0)
No	17 (60.7)
Don't know	4 (14.3)
Availability of any advertising rules for children's food packaging in your country (28)	
Yes	12 (42.9)
No	13 (46.4)
Don't know	3 (10.7)
Availability of any programs or interventions from the government to prevent obesity in children in your country (28)	
Yes	18 (64.3)
No	9 (32.1)
Don't know	1 (3.6)
Availability of any programs or interventions from schools to prevent obesity in children in your country (28)	
Yes	15 (53.6)
No	10 (35.7)
Don't know	3 (10.7)

COVID-19: Coronavirus disease-2019

Table 3. Non-governmental programs related to obesity in children

Characteristics (number)	Number of responses (%)
Presence of support of private organizations or non-profit organizations (civil society organizations) for childhood obesity prevention programs in your country (28)	
Yes	20 (71.4)
No	4 (14.3)
Don't know	4 (14.3)
If yes, are these programs nationally or locally operated? (20)	
Nationally operated	2 (10)
Locally operated	3 (15)
Both	15 (75)
Awareness of regional programs established to prevent childhood obesity (28)	
Yes	16 (57.1)
No	12 (42.9)
Awareness of WHO's guidance concerning childhood obesity (28)	
Yes	22 (78.6)
No	6 (21.4)
If "Yes", how has this guidance influenced your country's national programs? (20)	
Emphasizing childhood obesity as a priority	1 (5)
Adopting as references to develop national policies and regulations aimed to reduce obesity	7 (35)
Apply practical recommendations to national programs	3 (15)
Increase the awareness of childhood obesity	1 (5)
Participate in the WHO Europe Childhood Obesity Surveillance Initiative	1 (5)
Develop a Center for the Prevention and Treatment of Obesity in Children and Adolescents	1 (5)
Programs and regulations exist but hindered by poor compliance	3 (15)
Don't know	3 (15)
Awareness of UNICEF's guidance concerning childhood obesity (28)	
Yes	14 (50)
No	14 (50)
If "Yes", how has this influenced your country's national programs? (14)	
Emphasizing childhood obesity as a priority	1 (7.1)
Campaign and advocacy work	2 (14.3)
Adopting as references to develop national policies and regulations	4 (28.6)
Cooperation with the Chamber of Commerce and their support for obesity prevention	1 (7.1)
Increase the awareness of childhood obesity	2 (14.3)
Apply practical recommendations to national programs	3 (21.4)
Don't know	1 (7.1)

WHO: World Health Organization, UNICEF: The United Nations International Children's Emergency Fund

Table 4. Suggestions to overcome obesity in children

Characteristics (number)	Number of responses
Prevention is key at all ages	4
Recognize obesity as a disease, which prompts early diagnosis and regular check-ups to be mandatory	3
Holistic and comprehensive approach involving all stakeholders	5
Educate and empower families at the community level	9
Develop, support and reinforce regulations and policies that support a healthy food environment, including regulating processed foods to limit added sugar	11
Build national programs including providing weight management services	7
Facilitate exercise and promote physical activity across all ages	10
Encourage healthy habits from infancy by promoting breastfeeding	5
Involving schools as a key stakeholder	8
Raise public awareness of obesity by ensuring better access to information	3
Prevention begins with pre-pregnancy and pregnancy women	2
Encourage healthy eating habits	8
Tensions between for-profit and public health agendas must be acknowledged and addressed	1
Limit screen time	1
Encourage sleep at night	1
Support families to reduce divorce	1

influenced the cooperation with the Chamber of Commerce and their support for obesity prevention.

Lastly, the questionnaire asked for suggestions from participants to overcome obesity in children, summarized in Table 4. Whilst each participant gave several answers, the suggestions can be summarized as follows. The majority of responses centered on developing, supporting and reinforcing regulations and policies that support a healthy food environment (n = 11), facilitating exercise and promoting physical activity across all ages (n = 10), and educating and empowering families at the community level (n = 9). Other common answers included encouraging healthy eating habits (n = 8), the involvement of schools as crucial stakeholders (n = 8) and building national programs (n = 7). Participants also emphasized that prevention needed to be done at all ages (n = 4), beginning in infancy by promoting breastfeeding (n = 5) and in pre-pregnancy and pregnant women (n = 2). Several participants also highlighted that obesity needed to be properly recognized as a disease (n = 3) and that a holistic and comprehensive approach must involve all stakeholders (n = 5). Other answers included raising public awareness by improving access to information (n = 3), addressing tensions between for-profit and public health agendas (n = 1), limiting screen time (n = 1), encouraging sleep at night (n = 1) and supporting families to reduce divorce (n = 1).

Discussion

This study was conducted to identify the availability of national programs focusing on the prevention of obesity in the pediatric population, including regulations on children's food environment. Data collected from 28 countries across global regions, provided preliminary insights into different practices in implementing prevention measures against childhood obesity and, more importantly, multinational suggestions for improvement from pediatric societies' perspectives.

Given the emergency posed by the implications of the rising number of children with excessive weight globally, the aim of "No increase in childhood overweight" was endorsed by member states of WHO at the 65th World Health Assembly as one of six Global Nutrition Targets 2025 in the "Comprehensive Implementation Plan for Maternal, Infant, and Young Child Nutrition" (7). National prevalence data of obesity in children, one of the key indicators for monitoring, were reported available by more than three-quarters of participants from 22 different countries, although, four participants reported none, and one did not know. Compared to the latest "UNICEF-WHO-The World Bank: Joint Child Malnutrition Estimates", only one country among these five has "no data", defined as no input data (e.g., household survey data) for use in the country-level models (8). This discrepancy might be caused by low awareness and probably suggests that childhood obesity might not be highlighted or recognized as a top health issue.

The survey was undertaken when the epidemic of childhood obesity overlapped with the COVID-19 pandemic. Compared with the rate before the COVID-19 pandemic, increased weight gain in children and adolescents was documented in studies from China, Europe, and the USA (9). Most participants (60.7%) also noticed an increasing number of children with obesity in their countries. School closures, changes in children's dietary intake, physical inactivity, and unhealthy commodity industries' marketing strategies are among many consequences of pandemic mitigation policies causing this trend (10).

Obesity and overweight are complex problems. Given their multifaceted determinants and health consequences, multisectoral and comprehensive strategies are required to effectively and sustainably prevent and manage this problem. The food environment is one of the key drivers of the rise in obesity. It features increasing availability, affordability, and accessibility, including the marketing of foods high in saturated fats, trans-fats, sugars, and/or salt and these are usually highly processed (10-12).

Despite no details being provided on which food label system was implemented due to the use of close-ended survey questions, our initial finding shows that in most of the participants' countries, general information on the amount of sugar and salt was available in food products for children. The Chilean system is still considered a gold standard among multiple food labelling systems available globally. Octagonal black labels are used in their front-of-package labelling system for foods high in sugar, sodium, and saturated fats. Additionally, products with these labels are also banned from schools. A positive impact on knowledge and awareness and reductions in the consumption of unhealthy foods (13) resulting from providing simplified nutritional information should be considered as a policy to prevent obesity and encourage children to make healthier food choices.

Exposure to the child-targeted marketing of unhealthy foods plays a significant role in the development of childhood obesity (14,15). A set of recommendations was released by WHO in 2010, calling for international and national action to reduce the impact by restricting the marketing of foods and beverages high in saturated fats, trans-fatty acids, free sugar, or salt to children (14). Advertising rules for children's food

packaging were only reported available by 12 participants (42.9%) in their countries, while 13 reported none, and the rest did not know. Recent evidence has indicated that unhealthy food marketing increased children's dietary intake and preference (16) and influenced children's thoughts and behavior, particularly enhancing preference for, attitudes to and consumption of unhealthy marketed foods (15), further emphasizing the urgent need for food advertising restriction for foods marketed at children.

A systematic review of 153 randomized controlled trials in various settings, including schools, has documented provided evidence that combinations of diet and physical activity interventions can reduce the risk of obesity in young children (0 to 5 years), while interventions focusing only on physical activity will only reduce the risk of obesity in the age group 6-12 years and 13-18 years with some evidence that combination of interventions may be effective in these age groups (17). Our findings suggest that programs or interventions from the government and schools to prevent obesity in children are generally available, as reported by more than half of the participants, although not specified.

It is well established that a multi-sectoral and multidisciplinary approach is needed to tackle the childhood obesity epidemic. Current prevention models actively engage and involve representatives from multiple sectors, including private organizations, public health agencies and non-governmental organizations, including WHO and UNICEF. Most participants (71.4%) reported the availability of support from private organizations for childhood obesity prevention programs. Most of these programs were noted to be locally and nationally operated (75%).

WHO has placed substantial focus on preventing and managing childhood obesity. Beginning in 2012 with the adoption of the Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition by the World Health Assembly, WHO continued putting childhood obesity as a global priority by incorporating the prevalence of overweight in children under five as one of the indicators of the SDGs for nutrition (indicator 2.2.2) (7). With the establishment of the Commission on Ending Childhood Obesity in 2014, six recommendations were released by WHO in 2016 (18). These recommendations included the implementation of programs that promote the intake of healthy foods, reduce the intake of unhealthy foods, promote physical activity, reduce sedentary behaviors, and promote healthy school environments, integrate guidance for NCD prevention from preconception and antenatal care and provide family-based, multi-component, lifestyle weight management services (18). Most participants reported that they were aware of WHO's guidance concerning childhood obesity (78.6%). Participants further elaborated that guidance published by WHO had influenced their respective country's programs, with several participants reporting that comprehensive national programs based on WHO's recommendations have been implemented (15%), while others reported that the recommendations were used as a reference in developing national policies and regulations (35%). Our study found that implementing programs and regulations in several countries was hindered by poor compliance, as reported by 15% of our participants. However, further studies are needed to investigate specific regulations and programs implemented in each country, as well as factors affecting the success of such programs.

Similar to the WHO, UNICEF regional and country offices and headquarters have also begun taking action on preventing overweight in children and adolescents. Established in 2015, SDG No. 3.4 puts reduction of one third premature mortality from NCDs as a global priority (19). In 2016, a global meeting with internal and external stakeholders was conducted to advise UNICEF on the key focus of UNICEF programs concerning overweight children and adolescents (20). The year after, the prevention of overweight children and adolescents was incorporated into the UNICEF Strategic Plan 2018-2021 for the first time as part of Goal Area 1: Every child survives and thrives (21). The UNICEF Nutrition Strategy Framework 2020-2030 brought about the vision of "nutrition for every child" (22). With the goal of protecting and promoting diets, services and practices that support optimal nutrition, growth and development of all children, adolescents and women, the Strategy calls for a systems approach to maternal and child nutrition that builds on UNICEF's strengths as a multisectoral agency with partnerships across the world (22). Compared to our participants' awareness of WHO guidance on childhood obesity, fewer participants were aware of UNICEF's guidance concerning childhood obesity (50%). Those who were aware shared similar responses with the previous question on WHO's guidance regarding how UNICEF's guidance has influenced their country's programs.

The final section of the questionnaire explored suggestions from participants regarding how to tackle the childhood obesity epidemic. Each participant provided multiple suggestions, and thus there were more suggestions than the number of participants involved in this study. Amongst the suggestions received, most participants emphasized the importance of developing, supporting and reinforcing regulations that build a healthy food environment for children and families. Existing initiatives in the field of global health have shown that the success of programs are highly dependent on the ability to engage stakeholders

from multiple sectors including public health agencies (23). As reported in the WHO Global Nutrition Policy Review 2016-2017, only 78% of the 167 countries involved included nutrition-relevant policies, strategies and plans in their responses to address overweight children (24). A smaller percentage of countries had regulations on the marketing of complementary foods and marketing of food and non-alcoholic beverages to children, at 16% and 40% respectively (20).

Regulations and policies that address childhood overweight and obesity take many forms, ranging from policy interventions that support the creation of an enabling environment that promotes physical activity to formation of a cross-governmental task force that oversaw commercial investments, school education, nutrition labelling or taxation of sugary drinks (25). A systematic review found that for every 10% increase in sugary drinks price with a tax, sugary drink consumption is estimated to be reduced by 7% (26). Studies like this provide evidence that the involvement of public agencies through policies and regulations implemented on a national level have provided promising contributions to reducing childhood overweight and obesity.

Other common suggestions from participants included facilitating physical activity and encouraging healthy eating habits across all ages, especially through the involvement of schools as a key stakeholder. It has been well established that childhood obesity occurs due to an imbalance between energy intake and expenditure. Davison and Birch (27) described an ecological model that suggests that risk factors for childhood obesity include dietary intake, physical activity, and sedentary behavior, with environmental factors such as school policies, demographics and parenting style, acting as further influences on children's eating and activity habits. Studies reported that adolescents associate junk food with pleasure, independence and convenience, whilst viewing liking healthy food as "odd" (28). This supports the need for changing societal perceptions of eating behavior. In contrast, a high degree of physical exercise is linked to favorable impacts on bone mineral density, muscular growth, and metabolic profile (29,30). The WHO recommends that children should engage in physical activity for a minimum of 60 minutes per day. Unfortunately, it is estimated that only 20% of youth achieve this level of physical activity (31).

As children spend the majority of their time in schools, institutions of education play an essential role in promoting healthy eating behaviors as well as increasing physical activity. It is estimated that children consume a large proportion (19-50%) of their total daily calories at school (32). Evidence has also found that a school environment that facilitates physical activity is linked with a lower risk of overweight and obesity. When schools have at least three physical activity-friendly environmental factors, such as larger school size, more physical activity programs, and better physical activity teaching experience, students have a lower prevalence of obesity than those without (33,34). As children engage with various individuals throughout their lives, including healthcare professionals, caregivers and teachers, there is a need for continued training and education across sectors to ensure that all stakeholders are conscious of their interactions with children to minimize bias and stigma (35). In particular, teachers need to actively ensure that all children, regardless of their weight, can be included and are welcomed in all school activities (35). Linking with the importance of physical activity in school, teachers should be wary of inadvertently creating stigmatizing situations, for example by specifically targeting and excluding children with overweight or obesity from performing drills or tasks, that might expose them to additional teasing or bullying from their peers (35). Furthermore, schools should include specific policies concerning weight-based teasing in their anti-bullying policies (35).

Childhood and adolescent obesity prevention should occur at all ages, emphasizing the importance of not only the first 1000 days of life, but also the first 1000 weeks of life, beginning as early as in pre-pregnancy and pregnant women (36). It has been established that interventions in the pre-conception period help mitigate against the critical, life-long adverse effects of obesity which commence *in utero* with epigenetic effects, gene expression and function along with metabolic programming relating to appetite and energy expenditure and ongoing transgenerational effects (37). Furthermore, evidence has emphasized the protective action of breastfeeding against obesity compared with formula feeding, exclusive breastfeeding until 6 months of age and parental weight control before conception and during pregnancy (38,39). Recognizing the substantial influence of parents' practices and lifestyle on their children, it is important that parents and families are actively involved and educated regarding the prevention of childhood obesity. These are all suggestions that were raised by participants in the present study.

Our data demonstrated that although the majority of countries represented by participants involved have data and programs concerning childhood obesity, as well as existing support from private organizations, there is still room for improvement for the awareness and implementation of programs. The success of programs are still hindered by lack of compliance and public awareness. In order to ultimately achieve SDGs targets 2 and 3, united commitment and

action from all countries to conduct country-specific evaluation and interventions are needed to tackle childhood obesity globally.

Study Limitations

The number of participants in the survey disseminated was lower than initially expected. We hypothesize that this was as a result of several factors, including a short survey period and insufficient promotion to member societies to participate in the survey, as well as the survey being directed towards presidents of IPA member societies who might not necessarily have direct access to pediatricians with expertise in NCDs and obesity. With a lower response rate, the distribution of participants may be more restricted. Thus, insights from several regions may not have been represented in the study. Specifically, insights from low-middle income countries, where availability and support may be lower are less represented in this study. Overall, this highlights the need for future data collection to be more representative.

Conclusion

Despite the wide availability of national data on obesity in children and food labels containing information on the amount of sugar and salt, there are different practices in the implementation of prevention measures against childhood obesity globally, particularly in statutory regulation on food advertising and national programs. Whilst support from non-profit organizations and awareness regarding WHO and UNICEF guidance on childhood obesity is relatively high, its implementation is still hindered by a general lack of awareness and poor compliance. With the continuing worsening of the childhood obesity epidemic, it is time for stricter regulations on proven risk factors of obesity in children, enforced by country or regional legislation. Country and region-specific interventions are needed to ensure better awareness, compliance and hence, progress towards tackling the childhood obesity epidemic.

Ethics

Ethics Committee Approval: Ethical clearance was not required for this study.

Informed Consent: Cross-sectional electronic survey.

Authorship Contributions

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References

1. World Health Organization. Non-communicable Diseases: Progress Monitor 2017. Geneva (CH), World Health Organization, 2017.
2. Obesity and Overweight. World Health Organization (WHO). Cited: 13.04.2023. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
3. Lange SJ, Kompaniyets L, Freedman DS, Kraus EM, Porter R; DNP3; Blanck HM, Goodman AB. Longitudinal Trends in Body Mass Index Before and During the COVID-19 Pandemic Among Persons Aged 2-19 Years - United States, 2018-2020. MMWR Morb Mortal Wkly Rep 2021;70:1278-1283.
4. United Nations Children's Fund. The state of the world's children 2019: Children, food and nutrition, growing well in a changing world. New York (US), UNICEF, 2019.
5. Downs SM, Ahmed S, Fanzo J, Herforth A. Food Environment Typology: Advancing an Expanded Definition, Framework, and Methodological Approach for Improved Characterization of Wild, Cultivated, and Built Food Environments toward Sustainable Diets. Foods 2020;9:532.
6. Global Kids Food & Beverages Industry Market Reports. ReportLinker. Cited: 16.04.2023. Available from: https://www.reportlinker.com/p05864286/Global-Kids-Food-and-Beverages-Industry.html
7. World Health Organization. Comprehensive implementation plan on maternal, infant and young child nutrition. Geneva (CH), World Health Organization, 2012.
8. World Health Organization (WHO). Global database on child health and malnutrition. UNICEF-WHO-The World Bank: Joint child malnutrition estimates - Levels and trends. Geneva, WHO, 2019. Last Accessed Date: 01.04.2023. Available from: https://www.who.int/publications/i/item/9789240025257
9. Jebeile H, Kelly AS, O'Malley G, Baur LA. Obesity in children and adolescents: epidemiology, causes, assessment, and management. Lancet Diabetes Endocrinol 2022;10:351-365. Epub 2022 Mar 3
10. Di Cesare M, Sorić M, Bovet P, Miranda JJ, Bhutta Z, Stevens GA, Laxmaiah A, Kengne AP, Bentham J. The epidemiological burden of obesity in childhood: a worldwide epidemic requiring urgent action. BMC Med 2019;17:212.
11. Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL. The global obesity pandemic: shaped by global drivers and local environments. Lancet 2011;378:804-814.
12. World Health Organization (WHO). Protecting children from the harmful impact of food marketing: policy brief. Available from: https://www.who.int/publications/i/item/9789240051348

13. Rodríguez Osiac L, Cofré C, Pizarro T, Mansilla C, Herrera CA, Burrows J, Castillo C. Using evidence-informed policies to tackle overweight and obesity in Chile. Rev Panam Salud Publica 2017;41:e156.
14. World Health Organization. Set of Recommendations on the Marketing of Foods and Non-alcoholic Beverages to Children. WHO: Geneva, 2010
15. Smith R, Kelly B, Yeatman H, Boyland E. Food Marketing Influences Children's Attitudes, Preferences and Consumption: A Systematic Critical Review. Nutrients 2019;11:875.
16. Sadeghirad B, Duhaney T, Motaghipisheh S, Campbell NR, Johnston BC. Influence of unhealthy food and beverage marketing on children's dietary intake and preference: a systematic review and meta-analysis of randomized trials. Obes Rev 2016;17:945-959. Epub 2016 Jul 18
17. Brown T, Moore TH, Hooper L, Gao Y, Zayegh A, Ijaz S, Elwenspoek M, Foxen SC, Magee L, O'Malley C, Waters E, Summerbell CD. Interventions for preventing obesity in children. Cochrane Database Syst Rev 2019;7:CD001871.
18. United Nations. Transforming our world: The 2030 agenda for sustainable development. New York (US), United Nations, 2015.
19. World Health Organization. Report of the commission on ending childhood obesity. Geneva (CH), World Health Organization, 2016.
20. United Nations Children's Fund. Designing a UNICEF strategy for child overweight and obesity. Workshop report, 2016.
21. United Nations Children's Fund. UNICEF Strategic Plan 2018-2021. New York (US), United Nations Children's Fund, 2018
22. United Nations Children's Fund. UNICEF Nutrition Strategy 2020-2030. New York (US), United Nations Children's Fund, 2020.
23. Addy NA, Shaban-Nejad A, Buckeridge DL, Dubé L. An innovative approach to addressing childhood obesity: a knowledge-based infrastructure for supporting multi-stakeholder partnership decision-making in Quebec, Canada. Int J Environ Res Public Health 2015;12:1314-1333.
24. World Health Organization. Global nutrition policy review 2016-2017: country progress in creating enabling policy environments for promoting healthy diets and nutrition. Geneva (CH): WHO, 2018.
25. Brennan LK, Brownson RC, Orleans CT. Childhood obesity policy research and practice: evidence for policy and environmental strategies. Am J Prev Med 2014;46:1-16.
26. Afshin A, Peñalvo JL, Del Gobbo L, Silva J, Michaelson M, O'Flaherty M, Capewell S, Spiegelman D, Danaei G, Mozaffarian D. The prospective impact of food pricing on improving dietary consumption: A systematic review and meta-analysis. PLoS One 2017;12:e0172277.
27. Davison KK, Birch LL. Childhood overweight: a contextual model and recommendations for future research. Obes Rev 2001;2:159-171.
28. Chapman G, Maclean H. "Junk food" and "Healthy food": Meanings of food in adolescent women's culture. J Nutr Educ 1993;25:108-113.
29. Janssen I, Leblanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phys Act 2010;7:40.
30. Fritz J, Rosengren BE, Dencker M, Karlsson C, Karlsson MK. A seven-year physical activity intervention for children increased gains in bone mass and muscle strength. Acta Paediatr 2016;105:1216-1224. Epub 2016 May 16
31. Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U; Lancet Physical Activity Series Working Group. Global physical activity levels: surveillance progress, pitfalls, and prospects. Lancet 2012;380:247-257.
32. Story M, Nanney MS, Schwartz MB. Schools and obesity prevention: Creating school environments and policies to promote healthy eating and physical activity. Milbank Q 2009;87:71-100.
33. Ip P, Ho FK, Louie LH, Chung TW, Cheung YF, Lee SL, Hui SS, Ho WK, Ho DS, Wong WH, Jiang F. Childhood Obesity and Physical Activity-Friendly School Environments. J Pediatr 2017;191:110-116. Epub 2017 Oct 4
34. Sallis JF, McKenzie TL, Beets MW, Beighle A, Erwin H, Lee S. Physical education's role in public health: steps forward and backward over 20 years and HOPE for the future. Res Q Exerc Sport 2012;83:125-135.
35. Weghuber D, Khandpur N, Boyland E, Mazur A, Frelut ML, Forslund A, Vlachopapadopoulou E, Erhardt É, Vania A, Molnar D, Ring-Dimitriou S, Caroli M, Mooney V, Forhan M, Ramos-Salas X, Pulungan A, Holms JC, O'Malley G, Baker JL, Jastreboff AM, Baur L, Thivel D. Championing the use of people-first language in childhood overweight and obesity to address weight bias and stigma: A joint statement from the European-Childhood-Obesity-Group (ECOG), the European-Coalition-for-People-Living-with-Obesity (ECPO), the International-Paediatric-Association (IPA), Obesity-Canada, the European-Association-for-the-Study-of-Obesity Childhood-Obesity-Task-Force (EASO-COTF), Obesity Action Coalition (OAC), The Obesity Society (TOS) and the World-Obesity-Federation (WOF). Pediatr Obes 2023;18:13024. Epub 2023 Apr 1
36. 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
37. Şanlı E, Kabaran S. Maternal Obesity, Maternal Overnutrition and Fetal Programming: Effects of Epigenetic Mechanisms on the Development of Metabolic Disorders. Curr Genomics 2019;20:419-427.
38. Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC; Lancet Breastfeeding Series Group. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 2016;387:475-490.
39. Patro-Gołąb B, Zalewski BM, Kołodziej M, Kouwenhoven S, Poston L, Godfrey KM, Koletzko B, van Goudoever JB, Szajewska H. Nutritional interventions or exposures in infants and children aged up to 3 years and their effects on subsequent risk of overweight, obesity and body fat: A systematic review of Systematic Reviews. Obes Rev 2018;19:1620. Epub 2016 Oct 17

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SHOX Variations in Idiopathic Short Stature in North India and a Review of Cases from Asian Countries

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What is already known on this topic?

The worldwide prevalence of *SHOX* variations in idiopathic short stature (ISS) varies from 2.5% to 15%. *SHOX* variants are one of the most common causes of ISS. *SHOX* deletions are the most common type of variation encountered worldwide.

What this study adds?

This study highlights the role and prevalence of *SHOX* variations in ISS in a North Indian population. In addition, a meta-analysis is presented which compiled findings from the last decade and provides an updated insight into the prevalence of *SHOX* variations in the whole Asian population, underscoring their potential as therapeutic targets in ISS patients.

Abstract

Objective: Short stature homeobox *(SHOX)* haploinsufficiency underlies idiopathic short stature (ISS) and Leri-Weill dyschondrosteosis. The worldwide prevalence of *SHOX* variations in ISS varies from 2.5% to 15.0%. The aim of this study was to assess the implication of *SHOX* variation in ISS in North Indians and compare this with other cases of *SHOX* variations from Asian population.

Methods: *SHOX* gene analysis was carried out by multiplex ligation-dependent probe amplification followed by Sanger sequencing in 54 patients with variable phenotypes. Comparison with other reports in a meta-analysis comprising the current study and 11 previous studies (n = 979) was performed.

Results: *SHOX* analysis resulted in 12.9% positivity (7.4% deletions and 5.5% duplications). *SHOX* association was seen significantly related to gender, with predominance in females (p = 0.047). Short arms and forearms were the only significantly associated trait seen in 51.9% of children. The overall prevalence of *SHOX* variation was 15.2% in Asians with ISS. No significant difference was found in geographical region-specific analysis.

Conclusion: This study summarises findings from the last decade and provides an updated picture of the prevalence of *SHOX* variations in Asians, emphasizing their potential as therapeutic targets in ISS patients. Further high quality, large investigations including functional validation is warranted to validate this association.

Keywords: Idiopathic short stature, *SHOX*, MLPA, Sanger sequencing, meta-analysis, prevalence

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Introduction

Idiopathic short stature (ISS) is defined as height which is more than two standard deviations (>2 SD) below the corresponding average height in a given age, sex, and population without features suggestive of specific causative disorders (1). ISS affects around 2.0% of children and is a genetically heterogeneous condition. With the advance in techniques, a battery of genes is known to be involved in longitudinal growth in human beings, but despite this, genetic causes constitute only a small proportion of cases of short stature (2). One of the genes closely associated with human short stature is the short-stature homeobox-containing gene (*SHOX*), alternatively known as pseudoautosomal homeobox-containing osteogenic gene (3). The importance of this gene is highlighted it is one of the most common genetic causes leading to short stature in children, with either isolated or familial short stature.

The location of *SHOX* is characteristic and is responsible for the variable phenotype. Being located over the tip of the short arms of both sex chromosomes X and Y inside the telomeric region of the pseudoautosomal region 1 (PAR1), named due to its presence over both these chromosomes (4). The location is also pivotal as genes in this area escape the process of lyonization. *SHOX* has been shown to function in a dose-dependent manner, that is loss-of-function variation which involves only a part of the gene, thus involving one of the *SHOX* alleles, technically known as haploinsufficiency, can lead to a wide variety of phenotypes and resulting in short stature of varying degrees (5). Furthermore, *SHOX* deletions are the most common type of variations encountered worldwide, known to account for 80.0% of all variations (6). These deletions can be of varying sizes and can involve either all or part of the gene or regions above and below it, which contain a regulatory enhancer region (7). Other types of variations reported include nonsense and missense sequence alterations. Most common variations are seen in exons 3 and 4, which are known to encode the functional part of this pivotal homeodomain (7).

In different studies from around the world, estimates of the presence of *SHOX* gene variation leading to ISS range from 2.5% to 15.0% (8,9,10,11). However, the prevalence of ISS in India due to *SHOX* variations is not known. In the last few decades, many studies have been done on the association between different gene variations and ISS. The prevalence of *SHOX* variation depends on ethnicity and selection of patients. Some studies from Asian populations have reported important frequencies in comparison to the worldwide populations. In the present study, individuals with ISS, with height <2 SD of population reference range height, with no identified cause were included. These selected individuals had detailed anthropometric evaluation, dysmorphism evaluation and skeletal survey by radiographs. Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing of *SHOX* exons 2-6 were done to identify deletions and sequence variations, respectively. In addition, a meta-analysis of other cases from Asia with *SHOX* variations was performed to estimate and evaluate the relationship between *SHOX* variation and ISS in these populations.

Methods

Subjects

This was a prospective study conducted in a tertiary hospital from July 2020 to March 2022, with approval from the Ethics Committee of the Postgraduate Institute of Medical Education & Research (PGIMER) (approval no: NK/6656/MS/315, date: 01.12.2020). A detailed physical examination of children with ISS was performed to look for dysmorphic features, such as micrognathia, high-arched palate, short forearm, cubitus valgus, short hands, characteristic Madelung deformity, dislocation of ulna at the elbow, short lower leg, bowing of tibia, genu valgus, short feet, scoliosis, and/or muscular hypertrophy. Rappold scoring was done for better delineation of the clinical expression in our cohort (12). The children included in the study were measured for the following anthropometric parameters using standardized techniques and instruments, in the growth laboratory/clinic: height (cm); weight (kg); sitting height (cm); and arm span (cm). Body mass index (BMI; kg/m^2) and arm span to height ratio, as well as upper segment to lower segment ratio, was also calculated. Written informed consent was obtained from each patient or their parents or legal guardians, in the case of minors after explaining the purpose and type of all procedures used.

Molecular Analysis

Genomic DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) for subsequent molecular analysis. *SHOX* analysis was performed using MLPA technique using a SALSA P018-G2 kit (MRC-Holland, Netherlands) as per the manufacturer's instructions. The entire coding region of the *SHOX* gene (exons 2-6) and splice junctions were sequenced by Sanger sequencing using an automatic sequencer, the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, California). Primer sequences are available upon request.

Meta-analysis

Meta-analysis of prevalence was done using the MedCalc software, version 20.111 (https://www.medcalc.org/). This study followed the PRISMA guidelines (13). To identify the relevant publications concerning *SHOX* gene variations in ISS in Asia, the databases PubMed, Scopus and Web of Science were investigated. The search string was a combination of the following keywords: "Idiopathic Short Stature", "*SHOX*", "deletions/duplications/variations", "copy number variations". All the studies published in last 10 years (2012-2022) were selected for this study. The included studies met the following eligibility criteria: region - Asia; period - last 10 years; language - English; and peer-reviewed publication. Included studies were original research articles, related to *SHOX* prevalence, variations and/or incidence, along with the number of individuals included. The authors independently assessed the risk of bias for the included studies, using the Newcastle-Ottawa Scale (NOS) tool for quality assessment (14).

Statistical Analysis

The strength of the association between *SHOX* variation and ISS in the Asian population was evaluated by calculating the odds ratio using the Mantel-Haenszel statistics method. A fixed/random-effect model was applied, along with a corresponding 95% confidence interval (CI). Fixed-effects models were used to assess the pooled prevalence of genes for results with low heterogeneity (I^2 ≤50%). Otherwise, random-effects models were applied for the analyses. MedCalc software was used for all data synthesis and statistical analyses. Chi^2 and I^2 statistics were used to calculate heterogeneity across individual studies and subgroups. Population bias was assessed by Funnel-plot analysis. In addition, subgroup analysis was performed based on ethnicity or regional variation between subgroups. All statistical analyses were performed by using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 22.0 Armonk, NY: IBM Corp.

Results

A total of 54 patients were enrolled. Of these, 5 (9.3%) were in the age group 1-5 years, 20 (37.0%) were in the age group 5-10 years, 26 patients (48.0%) were 10-15 years of age and 3 patients (5.5%) were above 15 years of age. The median (interquartile range) age of children in this study was 11years (8 years, 13 years). The study included 26 females (48.1%) and 28 males (51.9%). Phenotypic characteristics of enrolled patients are given in Table 1. Short upper limbs (arms and forearms) was the most consistent feature (28/54, 51.85%) and was more common than short lower limbs (24/54, 44.4%). Short upper limbs was a significant finding in the variation positive subjects (Table 1). Mean, SD and range for the measured anthropometric parameters among the study cohort is shown in Supplementary Table 1. Most of the anthropometric parameters analyzed using SPSS software represented a moderately skewed distribution (almost towards normal distribution for practical purposes) with a skewness index ranging between +1 to -1. Detailed physical growth and pubertal characteristics of study subjects who were positive for *SHOX* variants (n=7, 13%) are depicted in Table 1. Interestingly, four out of these

Table 1. Association of phenotypic features with *SHOX* mutation

Phenotypic characters	*SHOX* mutation		p
	Yes (n=7)	No (n=47)	
	n (%)	n (%)	
Height (SDS)	-3.1+0.7	-2.7+1.2	0.316
Weight (SDS)	-2.0+0.5	-1.8+1.3	0.669
BMI (SDS)	-0.6+0.3	-0.5+1.2	0.826
Male	1 (1.9%)	27 (50.0%)	**0.047**
Female	6 (11.1%)	20 (37.1%)	
Micrognathia	2 (50.0%)	2 (50.0%)	0.077
High-arched palate	0 (0.0%)	7 (100.0%)	0.576
Short arm and forearm	5 (17.6%)	23 (82.1%)	**0.024**
Cubitus valgus	1 (33.3%)	2 (66.7%)	0.346
Madelung deformity	1 (50.0%)	1 (50.0%)	0.245
Short leg and feet	5 (20.8%)	19 (79.2%)	0.250
Genu varum	1 (0.0%)	0 (0.0%)	0.130
Muscle hypertrophy	0 (0.0%)	0 (0.0%)	-

SDS: standard deviation score, BMI: body mass index

seven cases had initially presented to the endocrinology OPD of Department of Pediatrics and were advised growth hormone therapy. However, due to financial constraints or being lost to follow-up, growth hormone was not initiated in these children, except for one case (Patient 3) in whom a marked increase in height gain was evident [from -6.4 SD score (SDS) at 8 years to -3.3 SDS at 13 years].

Molecular Analysis Results

SHOX del/dup was found in 12.9% patients (7/54) using MLPA, including heterozygous deletion of exons in 4 (7.4%), and duplications in 3 (5.5%). MLPA results of positive patients are given in Table 2 and Supplementary Figure 1. In the family of Patient 1, her mother and 17-year-old elder sister were also short. Mother was 139 cm (-3.73 SD) and elder sister was 140.5 cm (-3.46 SD) in height; they also had the same heterozygous *SHOX* exon 4 deletion. Father (171 cm) was also tested but did not have the deletion. In the family of Patient 4, in whom a heterozygous *SHOX* exon 4 deletion was detected, the mother was 128.5 cm (-5.31 SD). The family however did not consent for further testing in the mother. There was an apparently healthy younger brother with no short stature. In the other five families, two families had no other offspring and the other three families had one more offspring each with no history of short stature. From the above, it is apparent that 1 out of 7 (14%) copy number variations was familial. Sanger sequencing revealed no sequence variations in any of the MLPA negative patients. In two female patients with heterozygous exon 4 deletion on MLPA, Sanger sequencing revealed no sequence variations, thus ruling out point mutation at the ligation site as the cause.

Clinical Characteristics

Clinical parameters of the seven patients with *SHOX* variants, their Rappold scores (Supplementary Table 2) and phenotypic features are given in Table 1.

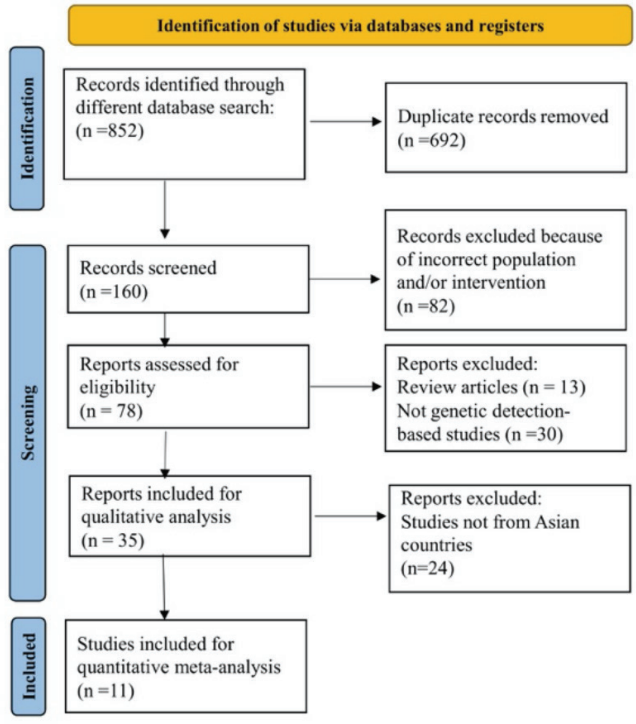

Figure 1. Flow chart for selection of studies (PRISMA 2020)

Table 2. Clinical and molecular features of the children with *SHOX* deficiency

	Age at enrolment and gender	Height	Weight	BMI	MPH	Father's height	Mother's height	Bone age	MLPA
Patient 1	14 years, female	137.1 cm (-2.7 SDS)	34.4 kg (-1.6 SDS)	18.3 (-0.3 SDS)	148.5 cm	171.0 cm	139.0 cm (mother was short)	Advanced	Heterozygous deletion of exon 4 of *SHOX* gene on Xp22
Patient 2	12 years, female	119.4 cm (-4.0 SDS)	25.0 kg (-2.3 SDS)	17.6 (-0.2 SDS)	154.5 cm	166.0 cm	156.0 cm	Normal	Heterozygous deletion of exon 5
Patient 3 (on GH therapy)	13 years, male	128.5 cm (-3.3 SDS)	28.0 kg (-2.0 SDS)	17.1 (-0.4 SDS)	164.2 cm	163.4 cm	152.0 cm	Delayed	Heterozygous deletion of exon 5, 6 of *SHOX* gene on Xp22
Patient 4	14 years, female	128.0 cm (-4.1 SDS)	27.0 kg (-2.8 SDS)	16.4 (-1.0 SDS)	138.0 cm	160.5 cm	128.5 cm (mother was short)	Normal	Heterozygous deletion of exon 4 of *SHOX* gene on Xp22
Patient 5	15 years, female	140 cm (-2.6 SDS)	35.5 kg (-1.7 SDS)	17.8 (-0.6 SDS)	157.5 cm	167.0 cm	161.0 cm	Delayed	Complete duplication of *SHOX* gene
Patient 6	12 years, female	128.5 cm (-2.9 SDS)	25.9 kg (-2.2 SDS)	15.8 (-0.9 SDS)	150.7 cm	162.0 cm	152.3 cm	Normal	Duplication of exons 1, 2, 3 including upstream region
Patient 7	8 years, female	110.6 cm (-2.4 SDS)	18.9 kg (-1.4 SDS)	15.6 (0.1 SDS)	152.6 cm	164.0 cm	154.1 cm	Normal	Duplication of downstream region of *SHOX* gene on Xp22

SDS: standard deviation score, GH: growth hormone, BMI: body mass index, MLPA: multiplex ligation-dependent probe amplification, *SHOX: Short-stature homeobox-containing gene*

Among the 54 cases, *SHOX* gene variation was present in 3.5% of boys and 23.1% of girls enrolled. This was a significant relationship between *SHOX* gene variations and female sex ($p = 0.047$) (Table 1).

From among all the traits which were noted among the enrolled patients, short arms and forearms were significant and related to those with ISS due to *SHOX* variations, whereas the other phenotypic characteristics were not found to be linked with variation. The phenotypic characters and their respective association are summarized in Table 1. No significant association was found for height, mid-parental height, weight, BMI, upper segment to lower segment ratio, or arm span to linear height ratio (data not shown). Radiological survey was done using X-ray of wrist, arms and forearm, legs, thoracolumbar spine and chest X-ray. However, no significant association was established between abnormal radiological findings and *SHOX* gene variation.

Meta-analysis

A total of 852 studies were retrieved. After the removal of duplicates (692 studies), 160 studies including the present study were considered potentially eligible for evaluation, but 149 did not meet the inclusion criteria, leaving 11 studies for analysis (Figure 1). This meta-analysis comprised 10 previous studies and the present study with a total of 979 participants. Detailed characteristics of the studies are provided in Table 3 with NOS scoring. Studies were conducted in six different Asian countries, which were further subcategorised into South, West and Eastern Asia. Most of the studies used MLPA as a major method, followed by Sanger sequencing, chromosomal microarray (CMA) and fluorescent *in situ* hybridisation analysis.

Variations in *SHOX* were identified in 83 of 979 patients. Using random effect model, the mean prevalence of *SHOX* variations was 14.4% (95% CI, 1.0-3.0, $p < 0.001$, $I^2 = 95.1\%$) (Figure 2A, Table 4). The Funnel-plot was asymmetric, suggesting the possibility of publication bias Figure 2B. Further prevalence and association were calculated on the basis of the different regions of Asia, divided into South Asia (patients n = 134), West Asia (n = 226) and East Asia (n = 619). No significant differences were observed in these rates in the different regions of the Asia. The mean frequency of *SHOX* variations in South Asia was 10.4%, with this rate being higher than the rates in other regions; East Asia (8.4%) and West Asia (7.5%) (Figure 3, Table 5).

Discussion

SHOX gene deficiency is one of the single gene disorders of bone metabolism resulting in highly variable osteodysplasia and so affecting the overall height of affected children and adults. However, as understanding of the gene and allelic alterations in the gene have evolved over time, it is now known that isolated deficiency of either the *SHOX* gene or its modifiers up and down the PAR region are responsible for a spectrum of disorders, ranging from simple ISS to more severe disorders, like Leri-Weill dyschondrosteosis (LWD, MIM 127300) and Langer Mesomelic dysplasia (LMD, MIM 249700)". In the present study, *SHOX* variants were found in 12.9% of patients using MLPA and Sanger sequencing, further sub-divided into heterozygous deletion of exons (n = 4), and duplications (n = 3).

In previous studies conducted earlier the prevalence of *SHOX* variations in children with ISS ranges from 2.0-15.0% (8,9,10,11). In a study by Hirschfeldova et al. (10), MLPA analysis detected *SHOX* gene anomalies in 13.7% (n = 7),

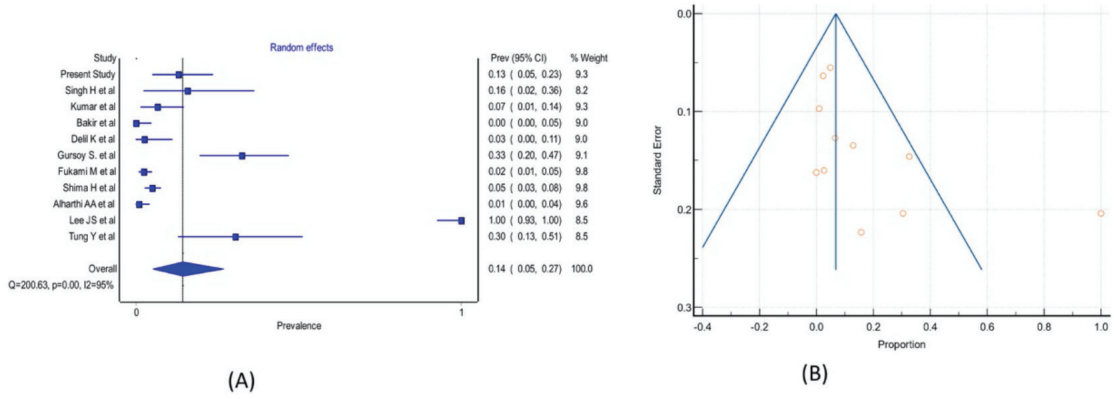

Figure 2. (A) The prevalence of *SHOX* variations is 14% (95% CI: 5.03-27.0, $p < 0.01$, $I^2 = 95\%$). (B) Funnel-plot for SHOX variation studies to analyse risk bias

CI: confidence interval

including 4 deletions, 1 duplication with an "ambiguous effect" and 2 *SHOX* gene point variations. In the same study, single isolated enhancer duplication was also observed in the LWD group. In the present study, three duplications were detected, with no apparent enhancer effect. In the present study, *SHOX* gene association was significantly ($p = 0.047$) related to gender, with predominance in female patients but there was no significant age correlation. Short arms and forearms were the only trait seen in 51.9% of the children and this was significantly ($p = 0.024$) related to *SHOX* gene variation, with the remaining phenotypic traits were not being significantly associated. The seven children with *SHOX* variants were investigated for phenotypic-genotypic correlation. Copy number changes involving the conserved non-coding DNA element (CNE) both upstream and downstream have been described in ISS (15). Duplications are very rare. In the present study, there were three heterozygous duplications, one involving the whole gene, one the first three exons and upstream and the third, downstream. All duplications gave rise to ISS. The transcriptional regulation of *SHOX* is highly complex. Genomic studies have identified multiple CNEs downstream of SHOX with there being a few upstream CNEs. CNEs have been reported to not always be highly conserved (5).

For better delineation of the clinical expression in our cohort, Rappold scoring was performed. Interestingly, the

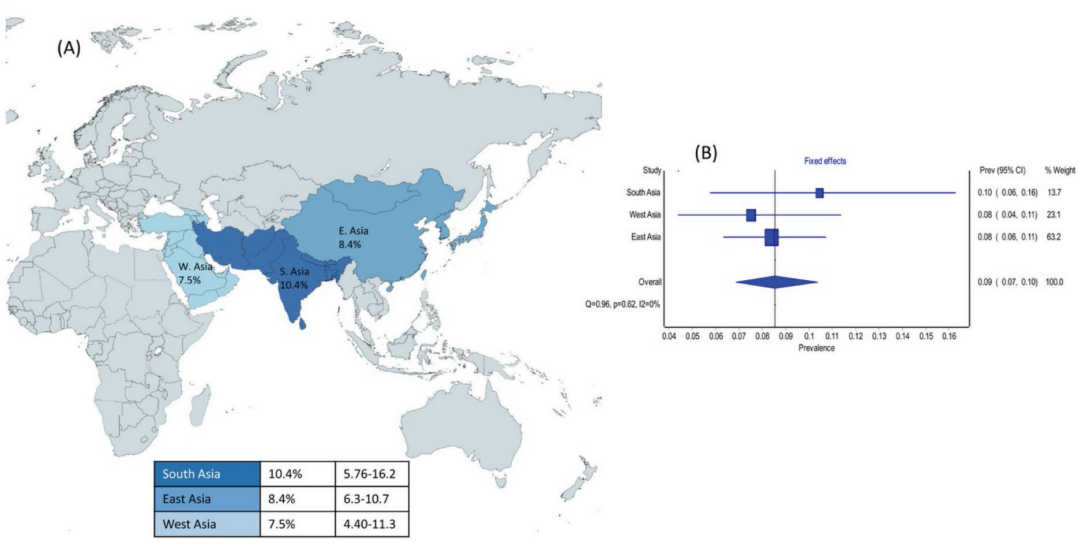

Figure 3. Prevalence of *SHOX* variations in ISS. (A) All studies were grouped according to the geographical origins of the patients. On the map of the world are shown the frequencies (%) of *SHOX* variations in South Asia, East Asia, and West Asia. (B) Dotted lines correspond to the overall prevalence and the 95% CI

ISS: idiopathic short stature, CI: confidence interval, I^2: inconsistency index

Table 3. Characteristics of studies included for analysis

Population	Country	Study	Positive cases/total cases/%	Methodology	NOS
South Asia	India	Present study, 2022	7/54/13%	MLPA and Sanger sequencing	7
		Singh et al. (19) 2018	3/19/15.8%	CMA	8
		Kumar et al. (18) 2020	4/61/6.6%	MLPA and Sanger sequencing	7
West Asia	Turkey	Delil et al. (20) 2016	1/38/2.6%	FISH	7
		Bakır et al. (21) 2018	0/37/0%	FISH and Sanger sequencing	7
		Gürsoy et al. (23) 2020	15/46/32.6%	MLPA and Sanger sequencing	7
	Saudi Arabia	Alharthi et al. (22) 2017	1/105/0.95%	Sanger sequencing	7
East Asia	Japan	Fukami et al. (5) 2015	6/245/2.45%	MLPA and CMA	7
		Shima et al. (26) 2016	16/328/4.9%	MLPA and CMA	7
	South Korea	Lee et al. (24) 2021	23/23/100%	MLPA, CMA and Sanger sequencing	7
	Taiwan	Tung et al. (25) 2018	7/23/30.4%	MLPA and Sanger sequencing	8

FISH: fluorescent *in situ* hybridisation, NOS: Newcastle-Ottawa Scale, MLPA: multiplex ligation-dependent probe amplification, CMA: chromosomal microarray

scores were very variable with a median (range) of 3 (0-10). Short forearm was the most commonly observed feature (62.5%), while cubitus valgus was observed in two patients. All reported BMIs were less than 50th percentile. Unexpectedly, abnormal arm span/height ratio was uncommon (28.5%), whereas bowing of forearm and tibia was present in only one patient. Sitting height/height ratio (>55.5%), dislocation of ulna at the elbow and muscular hypertrophy were absent in our cohort.

In different studies conducted in different populations, the prevalence of *SHOX* variations in ISS children again varies widely. Stuppia et al. (16) reported around 7.0% of patients with ISS having *SHOX* gene deletions, while

Table 4. Prevalence of *SHOX* mutation in Asian population along with test for heterogeneity and publication bias

Study	Prevalence (%)	LCI 95%	HCI 95%	Random effect weight (%)
Present study	12.9	0.1	0.2	9.3
Singh et al. (19) 2018	15.8	0.0	0.4	8.2
Kumar et al. (18) 2020	6.6	0.0	0.1	9.3
Bakır et al. (21) 2018	0.0	0	0.0	8.9
Delil et al. (20) 2016	2.6	0	0.1	8.9
Gürsoy et al. (23) 2020	3.3	0.2	0.5	9.1
Fukami et al. (5) 2015	2.4	0.0	0.0	9.8
Shima et al. (26) 2016	4.9	0.0	0.1	9.8
Alharthi et al. (22) 2017	1.0	0.0	0.0	9.6
Lee et al. (24) 2021	100.0	0.9	1.0	8.5
Tung et al. (25) 2018	3.4	0.1	0.5	8.5
Pooled	14.4	0.1	0.3	100.0
Test for heterogeneity				
Q	204.2			
DF	10			
Significance level	$p < 0.001$			
I^2 (inconsistency)	95.1%			
95% CI for I^2	92.9 to 96.6			
Publication bias				
Egger's test				
Intercept	5.4			
95% CI	-0.5 to 11.3			
Significance level	$p = 0.067$			
Begg's test				
Kendall's Tau	0.4			
Significance level	$p = 0.059$			

CI: confidence interval

Table 5. Prevalence of *SHOX* mutation in South, West and East Asian population

Study	Prevalence (%)	LCI 95%	HCI 95%	Fixed effect weight (%)
South Asia	10.4	0.1	0.2	13.7
West Asia	7.5	0.0	0.1	23.1
East Asia	8.4	0.1	0.1	63.2
Pooled	8.5	0.1	0.1	100.0
Statistics				
I-squared	0.0	0	78.3	
Cochran's Q	0.9			
Chi^2, p	0.6			

Musebeck et al. (17) investigated 35 patients with ISS, none of whom had the *SHOX* deletions.

This meta-analysis aimed to investigate the association between the *SHOX* variation and ISS in Asia. Data on 979 ISS cases was analysed. The overall prevalence of *SHOX* variation in Asia was 14.3%. Subgroup analysis showed the presence of *SHOX* variations in 10.4% of the patients with ISS in South Asia. Among South Asians, Kumar et al. (18) reported pathogenic heterozygous variants in 4 children (6.5%) out of 61, exon 5 duplication, splice site variation c.278-1G>C, one partial deletion and complete deletion of *SHOX*. Singh et al. (19) showed *SHOX* haploinsufficiency in two patients (10.5%), while one patient (5.2%) had mosaic gain in *SHOX* out of 19 patients.

In a West Asian population, Delil et al. (20) identified one patient (2.6%) with *SHOX* variation. In contrast, Bakır et al. (21) found no variation in the *SHOX* gene in 37 patients. Alharthi et al. (22) found only one variation in exon 4 of *SHOX* while rest of patients had polymorphisms in exons 1, 2, 4, and 6. Gürsoy et al. (23) found three point variations and one whole gene deletion in 15 patients from four different families. The overall prevalence of *SHOX* variants in ISS in West Asia was around 7.5%.

In Eastern Asia, Fukami et al. (5), reported six rare CNVs in PAR1 in 245 patients. Lee et al. (24), confirmed *SHOX* deficiency in 23 patients from 15 unrelated families. In a study by Tung et al. (25), *SHOX* intragenic deletions were found in five patients, one deletion in the regulatory region, and a missense variation at exon 5. Prevalence of variation in *SHOX* gene varied between different geographical regions of Asia, being highest in South Asia. Shima et al. (26) reported *SHOX* abnormalities in 3.8% of ISS and 50.0% of LWD cases. These results indicate the difference in the prevalence of the *SHOX* variations based on selection criteria, methodology, different sample size, and ethnicity.

Study Limitations

This was a hospital-based study and results should be interpreted in that context. Our study had a relatively small sample size, as it was a single centre study, and as it was a dissertation study, had to be concluded over a limited time period.

Conclusion

In the cohort of North Indian children with ISS, the prevalence of SHOX variants was 12.9%. This was consistent with the subgroup analysis of studies from this region. The meta-analysis, a compilation of findings from the last decade across western, southern and eastern Asia, presented an updated picture of overall prevalence of *SHOX* variations in Asians, underscoring its potential as a main target in ISS patients. Further investigations of higher quality, large cohort size with functional validation are warranted to validate this association.

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Ethics

Ethics Committee Approval: This was a prospective study conducted in a tertiary hospital from July 2020 to March 2022, with approval from the Ethics Committee of the Postgraduate Institute of Medical Education & Research (PGIMER) (approval no: NK/6656/MS/315, date: 01.12.2020).

Informed Consent: Written informed consent was obtained from the participants.

Authorship Contributions

Concept: Priyanka Srivastava, Saurabh Seth, Anupriya Kaur, Inusha Panigrahi, Devi Dayal, Subhodip Pramanik, Design: Priyanka Srivastava, Devi Dayal, Subhodip Pramanik, Data Collection or Processing: Ankita Tyagi, Chitra Bhardwaj, Anu Kumari, Harvinder Kaur, Saurabh Seth, Anupriya Kaur, Inusha Panigrahi, Devi Dayal, Subhodip Pramanik, Kausik Mandal, Analysis or Interpretation: Priyanka Srivastava, Ankita Tyagi, Chitra Bhardwaj, Anu Kumari, Saurabh Seth, Literature Search: Ankita Tyagi, Chitra Bhardwaj, Anu Kumari, Saurabh Seth, Writing: Priyanka Srivastava, Ankita Tyagi.

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References

1. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM; 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J Clin Endocrinol Metab 2008;93:4210-4217. Epub 2008 Sep 9
2. Rosenfeld RG, Hwa V. Toward a molecular basis for idiopathic short stature. J Clin Endocrinol Metab 2004;89:1066-1067.
3. Rao E, Blaschke RJ, Marchini A, Niesler B, Burnett M, Rappold GA. The Leri-Weill and Turner syndrome homeobox gene SHOX encodes a cell-type specific transcriptional activator. Hum Mol Genet 2001;10:3083-3091.
4. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth

failure in idiopathic short stature and Turner syndrome. Nat Genet 1997;16:54-63.

5. Fukami M, Naiki Y, Muroya K, Hamajima T, Soneda S, Horikawa R, Jinno T, Katsumi M, Nakamura A, Asakura Y, Adachi M, Ogata T, Kanzaki S; Japanese SHOX study group. Rare pseudoautosomal copy-number variations involving SHOX and/or its flanking regions in individuals with and without short stature. J Hum Genet 2015;60:553-556. Epub 2015 Jun 4
6. Benito-Sanz S, del Blanco DG, Aza-Carmona M, Magano LF, Lapunzina P, Argente J, Campos-Barros A, Heath KE. PAR1 deletions downstream of SHOX are the most frequent defect in a Spanish cohort of Léri-Weill dyschondrosteosis (LWD) probands. Hum Mutat 2006;27:1062.
7. Schneider KU, Marchini A, Sabherwal N, Röth R, Niesler B, Marttila T, Blaschke RJ, Lawson M, Dumic M, Rappold G. Alteration of DNA binding, dimerization, and nuclear translocation of SHOX homeodomain mutations identified in idiopathic short stature and Leri-Weill dyschondrosteosis. Hum Mutat 2005;26:44-52.
8. Huber C, Rosilio M, Munnich A, Cormier-Daire V; French SHOX GeNeSIS Module. High incidence of SHOX anomalies in individuals with short stature. J Med Genet 2006;43:735-739. Epub 2006 Apr 5
9. Jorge AA, Souza SC, Nishi MY, Billerbeck AE, Libório DC, Kim CA, Arnhold IJ, Mendonca BB. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. Clin Endocrinol (Oxf) 2007;66:130-135.
10. Hirschfeldova K, Solc R, Baxova A, Zapletalova J, Kebrdlova V, Gaillyova R, Prasilova S, Soukalova J, Mihalova R, Lnenicka P, Florianova M, Stekrova J. SHOX gene defects and selected dysmorphic signs in patients of idiopathic short stature and Léri-Weill dyschondrosteosis. Gene 2012;491:123-127. Epub 2011 Oct 14
11. Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. Horm Res Paediatr 2011;75:81-89. Epub 2011 Feb 4
12. Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, Quigley CA, Ross JL, Niesler B. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. J Med Genet 2007;44:306-313. Epub 2006 Dec 20
13. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2019;339:b2700.
14. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Last Accessed Date: 2611.2022. Available from: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
15. Bunyan DJ, Gevers E, Hobbs JI, Duncan-Flavell PJ, Howarth RJ, Holder-Espinasse M, Klee P, Van-Heurk R, Lemmens L, Carminho-Rodrigues MT, Mohamed Z, Goturu A, Hughes CR, Ajzensztejn M, Thomas NS. Rare dosage abnormalities flanking the SHOX gene. Egypt J Med Hum Genet 2021;22:89.
16. Stuppia L, Calabrese G, Gatta V, Pintor S, Morizio E, Fantasia D, Guanciali Franchi P, Rinaldi MM, Scarano G, Concolino D, Giannotti A, Petreschi F, Anzellotti MT, Pomilio M, Chiarelli F, Tumini S, Palka G. SHOX mutations detected by FISH and direct sequencing in patients with short stature. J Med Genet 2003;40:11.
17. Musebeck J, Mohnike K, Beye P, Tönnies H, Neitzel H, Schnabel D, Grüters A, Wieacker PF, Stumm M. Short stature homeobox-containing gene deletion screening by fluorescence in situ hybridisation in patients with short stature. Eur J Pediatr 2001;160:561-565.
18. Kumar A, Jain V, Chowdhury MR, Kumar M, Kaur P, Kabra M. Pathogenic/likely pathogenic variants in the SHOX, GHR and IGFALS genes among Indian children with idiopathic short stature. J Pediatr Endocrinol Metab 2020;33:79-88.
19. Singh H, Tiwari P, Bhavi V, Chaudhary PS, Suravajhala P, Mohan MK, Mathur SK. Application of Chromosomal Microarray for Evaluation of Idiopathic Short Stature in Asian Indian Children: A Pilot Study. Indian J Endocrinol Metab 2018;22:100-106.
20. Delil K, Karabulut HG, Hacıhamdioğlu B, Şıklar Z, Berberoğlu M, Öçal G, Tükün A, Ruhi HI. Investigation of SHOX Gene Mutations in Turkish Patients with Idiopathic Short Stature. J Clin Res Pediatr Endocrinol 2016;8:144-149. Epub 2015 Dec 18
21. Bakır A, Yirmibeş Karaoğuz M, Perçin FE, Tuğ E, Cinaz P, Ergün MA. Microdeletion and mutation analysis of the SHOX gene in patients with idiopathic short stature with FISH and sequencing. Turk J Med Sci 2018;48:386-390.
22. Alharthi AA, El-Hallous EI, Talaat IM, Alghamdi HA, Almalki MI, Gaber A. Screening of SHOX gene sequence variants in Saudi Arabian children with idiopathic short stature. Korean J Pediatr 2017;60:327-332. Epub 2017 Oct 20
23. Gürsoy S, Hazan F, Aykut A, Nalbantoğlu Ö, Korkmaz HA, Demir K, Özkan B, Çoğulu Ö. Detection of SHOX Gene Variations in Patients with Skeletal Abnormalities with or without Short Stature. J Clin Res Pediatr Endocrinol 2020;12:358-365. Epub 2020 Apr 16
24. Lee JS, Kim HY, Lee YA, Lee SY, Cho TJ, Ko JM. Clinical and Genetic Characteristics of 23 Korean Patients with Haploinsufficiency of the Short-stature Homeobox-containing Gene. Exp Clin Endocrinol Diabetes 2021;129:611-620. Epub 2020 Sep 15
25. Tung YC, Lee NC, Hwu WL, Liu SY, Lee CT, Chien YH, Tsai WY. SHOX deficiency in short Taiwanese children: A single-center experience. J Formos Med Assoc 2018;117:909-914. Epub 2017 Dec 16
26. Shima H, Tanaka T, Kamimaki T, Dateki S, Muroya K, Horikawa R, Kanno J, Adachi M, Naiki Y, Tanaka H, Mabe H, Yagasaki H, Kure S, Matsubara Y, Tajima T, Kashimada K, Ishii T, Asakura Y, Fujiwara I, Soneda S, Nagasaki K, Hamajima T, Kanzaki S, Jinno T, Ogata T, Fukami M; Japanese SHOX study group. Systematic molecular analyses of SHOX in Japanese patients with idiopathic short stature and Leri-Weill dyschondrosteosis. J Hum Genet 2016;61:585-591. Epub 2016 Mar 17

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Minipuberty in Male Full-term Neonates Appropriate and Small for Gestational Age and in Preterm Babies: Data from a Single Centre

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What is already known on this topic?

Previous studies have shown hypothalamic-pituitary-gonadal axis activation during early life, also referred to as "minipuberty". This period has sex-related differences in levels and duration of both gonadotropins and sex steroid secretion. The role of minipuberty seems to be significant mainly for development and maturation of reproductive organs, particularly in males.

What this study adds?

This study provides a longitudinal analysis with serial samples and a between-group comparison in term and preterm (PT) infants. Our results suggest that minipuberty is increased and prolonged in PT boys only when the analysis depends on calendar age, suggesting an ontogenetic regulation. Full-term small for gestational age infants had the lowest postnatal testosterone levels with higher urinary gonadotropins levels.

Abstract

Objective: The postnatal activation of the hypothalamic-pituitary-gonadal (HPG) axis is usually known as "minipuberty". There are still open questions about its biological function and significance depending on sex, gestational age (GA) and birth weight (BW) with few available longitudinal data.

Methods: A single-centre, longitudinal study to quantify urinary follicle stimulating hormone (uFSH), luteinizing hormone (uLH) and testosterone (uTs) in male neonates. Neonates were enrolled and stratified into three subgroups: full-term boys appropriate for GA (FT AGA); FT boys with BW ≤3rd centile [FT small for gestational age (SGA)]; and preterm (PT) boys ≤33 weeks of GA. Urinary hormones were correlated to simultaneous auxological parameters, linear growth and external genitalia at scheduled time-points.

Results: Forty-six boys were recruited, with subgroup sizes FT AGA n = 23, FT SGA n = 11 and PT n = 12. PT boys display a pulsatile pattern of urinary gonadotropins (uGns) with higher levels of uLH and a gradual increase of uTs. Testicular descent started from 29-32 weeks with the peak of uTs. During the first 12-months post-term age (PTA), FT AGA boys displayed a better linear growth ($p < 0.05$). PT showed higher uGns levels until 3-months PTA. PT babies had higher uLH levels than FT AGA, with a peak at 7 and 30 days, during the first 90 days of life ($p < 0.001$) and higher uTs levels. Correlation analysis between penile growth of all neonates and uTs was significant ($p = 0.04$) but not within subgroups.

Conclusion: This study investigated postnatal HPG axis activation in term and PT infants. Minipuberty may involve an early window of opportunity to evaluate the functionality of the HPG axis. Further studies with a long-term follow-up are needed with a special focus on possible consequences of GA and BW.

Keywords: Minipuberty, urinary gonadotropins, newborn, infants, prematurity, growth

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Introduction

Puberty is preceded by two periods marked with a transitional activation of the hypothalamic-pituitary-gonadal (HPG) axis: the first during foetal life, playing a crucial role in sex determination, the second during the first postnatal months, the second period of HPG activation is referred to as "minipuberty"(1), and its biological significance is still not completely understood.

Studies with moderate-sized cohorts of neonates have shown that minipuberty has sex-related differences (2) in levels and duration of both gonadotropin and sex steroid secretion. The gonadotropins start rising at around one week of age, peak (reaching pubertal levels) between one and three months of life, and decline to prepubertal values towards the age of six months (3,4,5,6,7). Male neonates have a luteinizing hormone (LH) peak higher than follicle stimulating hormone (FSH) levels with a gradual decrease to prepubertal levels at around 6-9 months (3,6). Testosterone (T) level starts to increase following the LH rise with a similar pattern (5,6,8). Both LH and T reach higher levels in boys than in girls (5,7,8,9,10). In contrast, female infants have a predominantly FSH level rise that remains elevated for a longer period, whereas estradiol (E) levels display a fluctuating pattern, probably reflecting the ovarian follicular cycle of growth and atrophy (1).

The role of minipuberty seems to be significant mainly for development and maturation of reproductive organs, particularly in males (11). In addition, androgens have also been related to other aspects of infant growth, such as cutaneous manifestations (12,13), linear growth, bone mineralization, psychosexual development and behaviour (14,15,16,17,18,19,20,21,22,23,24,25).

Little is known about the influence of factors such as birthweight or prematurity on the HPG axis. The HPG axis activation in babies born small for gestational age (SGA) is not well defined and its short-term and long-term effects on growth and development are still controversial. Studies on SGA females have found higher postnatal FSH levels compared with neonates born appropriate for gestational age (AGA). Meanwhile, in male SGA term neonates, HPG axis activation has been linked to both lower (26) and higher (27) gonadotropins and androgen (28) levels, with uncertain effects in adult life (29). In addition the impact of prematurity has been investigated mainly using cross-sectional studies based on serum samples (3,4,6) but only a few studies (1,6,7) have adopted a longitudinal approach. Based on recent longitudinal studies using urinary gonadotropins (uGns), preterm (PT) birth does not seem to influence the onset of postnatal HPG axis activation, as gonadotropin levels begin to rise with the same timing in full-term (FT) and PT infants (1). Moreover, minipuberty in PT babies seems to be stronger and prolonged with uncertain significance and effects (30,31,32) but data are still not univocal.

Understanding the physiological trend of hormonal levels during minipuberty and its differences between term, PT and SGA boys will therefore be helpful in understanding possible short-term and long-term effects.

The aims of the present study were to quantify urinary FSH (uFSH), urinary LH (uLH) and urinary testosterone (uTs) in male neonates, and to compare changes in timing and magnitude of uGns (uFSH and uLH) and uTs secretion in three different groups of neonates: FT boys appropriate for gestational age (GA) (FT AGA); FT boys small for GA (FT SGA); and PT boys ≤33 weeks of GA. Secondary aims were to make between-group comparisons of external genitalia clinical examination (penile length, testicular volume and position) and linear growth, with a focus on catch-up growth in FT SGA and PT infants.

Methods

Population

This was a single-centre, longitudinal and prospective study conducted on male neonates.

Neonates enrolled were split into three categories: FT AGA boys, FT SGA boys and PT boys born before 33 weeks of GA. Comparisons were made according to calendar age or post-term age (PTA). Neonates with antenatal genetic diagnosis or severe metabolic, cardiological, endocrine or sex development disorders (33) were excluded. Being classified as AGA or SGA among FT boys was based on birth weight (BW), as previously described (34). SGA was considered as a boy with a BW ≤3rd centile whereas all FT AGA boys had a BW > 10th centile. For PT babies, no classification according to BW was performed.

Medical data were collected from clinical records. Clinical assessments for FT neonates were: within the first 72 hours of life, at 1 week, at 1-3 and 6-12 months of life. For PT neonates evaluations were: within first 72 hours of life, once a week since term age, at 1-3 and 6-12 months of PTA. The number of evaluations depended on GA at birth and the child's clinical condition.

At each scheduled time-point, both clinical evaluation and urinary collection were performed. Clinical evaluation included auxological parameters [weight, length, head circumference (HC)] and external genitalia assessment (penile length, testicular position and volume).

Weight was measured with a digital baby scale to the nearest 5 g. The recumbent length was measured by a portable, high quality infantometer (GIMA, Baby Height Measuring Mat, cod. 27331) to the nearest 0.1 cm. HC was measured using a measuring tape from the most prominent part of the forehead around to the widest part of the back of the head. If the baby's condition was to poor for measurements among PT babies in the Neonatal Intensive Care Unit, both clinical evaluation and urine collection were postponed.

For PT babies, birth weight and the following growth till term age was monitored using national charts (34). For term babies, being AGA or SGA was assessed according to national charts (34). The international longitudinal charts World Health Organization 2006 (35) were used from 1- to 12-months PTA. National charts at birth and within term corrected age was preferred due to the single centre nature of the study. Furthermore, national charts within the first two years PTA are not available.

Penile length was measured as described by Boas et al. (11) and reported as a numeric value. The penis was slightly straightened and the distance between the lower edge of the pubic bone and the tip of the glans penis (excluding foreskin) was measured using a caliper.

All measurements were assessed by a trainee in Paediatrics and repeated twice to reduce errors. Testicular position was classified according to Boisen et al. (36) as "non-palpable", "inguinal" or "descended". Testicular volume was quantified using the Prader orchidometer.

Urine Collection and Assays

Urine samples were collected with a plastic bag (GIMA, cod.28685) made to fit baby's genital area.

Assays were ideally performed on fresh urine for: uFSH, uLH, uTs and urinary creatinine (uCr). When the analysis on fresh urine was not possible, urine samples were stored in a fridge at 4 °C and sent to the laboratory for the analysis within the next 24 hours. Each urine sample was analysed by the department of laboratory medicine and pathological anatomy of the University Hospital in Modena. uFSH and uLH were quantified with an electrochemiluminescence immunoassay "ECLIA" (Cobas E601, Roche Diagnostics, Rotkreuz, Switzerland). Lower limit of uGns detection was 0.1 mIU/mL, both for FSH and for LH. Intermediate precision was $<4.5\%$ and $<3.7\%$ at concentrations ranging from 0.65-152 mIU/mL for FSH, and $<2.2\%$ and $<1.6\%$ at concentrations ranging from 1.3-123 mIU/mL for LH. uTs was quantified with the same ECLIA. Lower and upper limits of uTs detection were 0.025-15 ng/mL, respectively. Intermediate precision was $<14.8\%$ and $<2.1\%$ at concentrations ranging from 0.063-14 ng/mL. The conversion factor for uTs is ng/mL x 3.47 = nmol/L. uCr was quantified using the certified VITROS 5600 Integrated System (Ortho Clinical Diagnostics). Lower and upper limits of uCr detection were 1.2-346.5 mg/dL, respectively. Intermediate precision was $<2.3\%$ and $<2.1\%$ at concentrations ranging from 61.7-157.5 mg/dL.

Both uGns and uTs values were also corrected for creatinine by dividing the uGns or uTs value for the corresponding creatinine content of each urine sample. However, due to the significant difference in uCr between term and PT infants, results have been interpreted only using non-corrected values.

Statistical Analysis

All data are expressed as median (range) or mean ± standard deviation. Due to missing samples, especially in PT babies, comparison of continuous variables was carried out using a mixed model analysis. Time (calendar age or PTA) and category of neonates were included in the model as fixed effects. Subject ID and twinness were included as random effects. Bonferroni adjustment was used for multiple comparisons. All results of hormone levels below or above the detection limits were considered as the lowest or highest values, respectively, to avoid zero or negative values. The average uTs during the first three months of age was calculated using the area under the curve with the trapezoidal rule and divided by the total time. Subsequently, the average hormone level was correlated with the penile and linear growth over the same period using Spearman's correlation. IBM Statistical Package for the Social Sciences statistics for Windows, version 26.0 (IBM Inc., Armonk, NY, USA) and Jamovi software (contact@jamovi.org) version 1.0.7.0 were used for statistical analysis. A $p<0.05$ was considered statistically significant.

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The AOU/AUSL Modena Local Ethics Committee approved the protocol (no: 329/17, date: 29.11.2017). Informed consent was obtained from all families. Data supporting these findings are available on request from the corresponding author only, due to privacy and ethical restrictions.

Results

Subjects

Data from 46 neonates were analyzed including: FT AGA n = 23 (50%); FT SGA n = 11 (24%); and PT neonates ≤33

weeks of GA n = 12 (26%). Most were Caucasian (94%) and 10/46 neonates (22%) were twins from diamniotic bichorial pregnancies. Maternal and obstetrical data, together with clinical information of male neonates, are described in Supplementary Tables 1 and 2, respectively.

There were nine (19%) babies lost to follow-up: FT AGA n = 4, FT SGA n = 4 and PT n = 1.

Comparison Between Groups Depending on PTA

Auxological parameters and external genitalia: Auxological parameters and penile length are shown in Table 1. The between-group comparison is shown in Figure 1.

At birth (or at expected term for PT infants) weight was higher in FT AGA neonates compared to FT SGA ($p < 0.001$) and PT ($p < 0.001$). This difference remained significant at 1, 3 and 6 months PTA only between FT AGA and FT SGA babies (respectively $p = 0.007$; $p = 0.013$; and $p < 0.001$). Moreover, no differences between FT SGA and PT boys were observed at any time-point.

Body length percentile was higher in FT AGA boys from birth to 6-months PTA, both when compared to FT SGA ($p < 0.001$) and to PT babies ($p < 0.001$).

No differences in penile length were found apart from a slightly divergence at 1-month PTA in which FT AGA and FT SGA had longer measurements in comparison with PT boys ($p = 0.03$). Testicular volume did not show any difference.

Urinary gonadotropins and urinary testosterone: Longitudinal trend of hormonal levels until 12-months PTA are shown in Figure 2. uFSH and uLH were significantly higher at the expected term in PT neonates in comparison with FT SGA ($p = 0.054$ and $p = 0.021$, respectively). Comparison between PT boys and FT AGA displayed higher level of uLH at expected term and 3-months PTA in the PT group ($p < 0.001$ and $p = 0.026$, respectively). However, uTs was significantly higher in FT AGA neonates both in comparison with FT SGA and PT babies ($p < 0.001$).

Linear Growth, External Genitalia Development and Hormone Levels in Preterm Infants

The longitudinal trend of linear growth in PT boys from birth to discharge showed a significant decrease during the hospital stay ($p < 0.05$).

Looking at the individual values of uGns and uTs, there was much variability among PT male neonates. Both uFSH and uLH displayed a pulsatile pattern starting within the first four weeks of life. Subsequently, uTs values also had a rise over this period. However, individual trends of duration and magnitude of this HPG axis activation over the first

Table 1. Auxological parameters [average (range)] for each category of male neonates: FT AGA (full-term AGA), FT SGA (full-term SGA) and PT (preterm). Data are shown from birth (presumed term of GA for PT boys) to 12 months of PTA. Significant differences in percentiles are marked as follow: °p < 0.05; ^p < 0.001; *p < 0.0001

	Birth/term of CGA			1 month PTA			3 months PTA			6 months PTA			12 months PTA		
	FT AGA	FT SGA	PT	FT AGA	FT SGA	PT	FT AGA	FT SGA	PT	FT AGA	FT SGA	PT	FT AGA	FT SGA	PT
Weight (gr)	3479 (2990-4150)	2456 (2180-2770)	2723 (1940-3240)	4366 (3380-5150)	3550 (3030-4700)	3932 (2940-5130)	6386 (5420-8100)	5648 (4420-6260)	5746 (4650-7170)	8295 (7010-10000)	6880 (5510-8000)	7379 (5970-8900)	9751 (8600-12000)	8504 (7000-9240)	8945 (7500-11000)
Weight (centile)	**58* (19-96)**	**1.8* (0-3)**	**15* (0-49)**	**40° (10-91)**	**10° (1-60)**	25 (1-80)	**47° (10-98)**	**18° (1-40)**	26 (2-80)	**60* (20-100)**	**13* (1-25)**	41 (2-80)	48 (20-100)	18 (2-40)	32 (5-75)
Lenght (cm)	50.8 (48-54)	47.5 (45-50)	46 (42-51)	55 (52-59)	51 (49-55)	51 (49-55)	62 (59-65)	58 (52-62)	59.4 57-61)	68 (65-71)	65 (61-69)	67 (63-71)	78 (72-80)	72.8 70-74)	75.5 (71-79)
Lenght (centile)	**59* (11-99)**	**13* (1-46)**	**15* (0-77)**	**56* (5-99)**	**12* (1-45)**	**15* (1-60)**	**60* (10-95)**	**13* (0-60)**	**15* (2-25)**	**57° (12-95)**	**20° (2-70)**	27 (0-50)	48 (10-90)	16 (0-25)	47 (3-75)
HC (cm)	33,3 (32-37)	32.7 (30-34)	34 (31-36)	37.7 (36-39.5)	36 (34-38)	38 (34.5-40)	41 (40-43)	40 (38-41)	41 (39-44)	44 (42-46.5)	42 (41-44)	43 (41-47)	47 (45-47.5)	45.4 (44-47)	46 (44.5-49.5)
HC (centile)	**55^ (2-97)**	**27^ (0-89)**	**49° (2-89)**	**66° (10-91)**	**35° (2-93)**	51 (9-91)	58 (20-95)	24 (1-60)	37 (9-98)	**48* (10-93)**	**13* (2-50)**	**28° (2-98)**	**52° (20-75)**	**12° (3-25)**	29 (5-95)
Penile lenght (mm)	27 (17-40)	28 (22-40)	27 (21-38)	33 (22-40)	34 (26-42)	27 (21-31)	30 (23-50)	33 (20-45)	30 (23-35)	32 (25-38)	33 (24-40)	29 (26-42)	38 (30-50)	39 (30-50)	39 (35-43)

PT: preterm, AGA: appropriate for GA, SGA: small for gestational age, HC: head circumference, FT: full-term, PTA: post-term age

two months of life was greatly variable between different subjects.

Average levels of uFSH and uLH displayed a pulsatile pattern with higher values of uLH, as expected in male neonates. uTs was high at birth with a subsequent decrease and a second rise just after the uLH peak at 3-4 weeks of life. Thereafter uTs levels remained high and testicular descent of PT babies was parallel to the uTs surge. Individual and mean values of urinary hormone levels are shown in Figure 3.

Figure 4 shows that PT babies had a postnatal HPG axis activation that was prolonged in its duration but also in its amplitude, in comparison with both groups of term neonates (AGA and SGA).

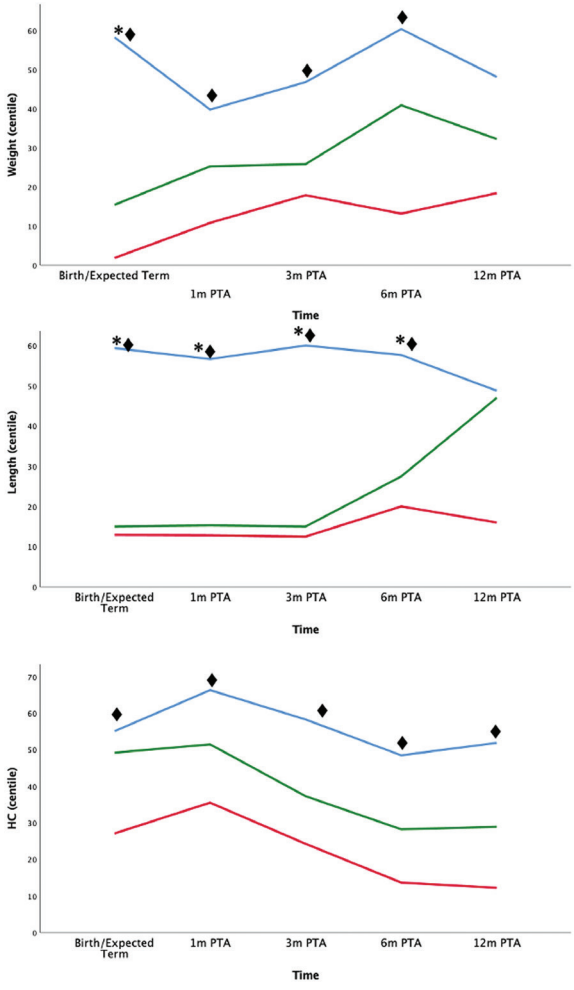

Figure 1. Linear trend of auxological and external genitalia parameters from birth (corresponding to the presumed term of GA for PT boys) to 12 months of post-term age. Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green). Differences statistically significant are indicated with this legend: *FT AGA≠PT, ♦FT AGA≠FT SGA, ◊FT SGA≠PT

GA: gestational age, PT: preterm, PTA: post-term age, AGA: appropriate for GA, SGA: small for gestational age, HC: head circumference

Comparison Between Groups Depending on Calendar Age

External genitalia: Penile length was higher in FT AGA and FT SGA neonates at birth, at one week and at 30 days of life ($p < 0.001$) compared to PT boys. Penile length was higher in SGA neonates at 90 days ($p = 0.028$).

Urinary gonadotropins and urinary testosterone: The mixed model analysis revealed a very different pattern in longitudinal assessment of urinary hormone levels between groups using calendar age during the first three months of life. At birth, uTs was higher in FT AGA neonates compared with PT and FT SGA babies ($p < 0.001$ and $p = 0.001$, respectively). At seven days of life, uLH had a peak at higher levels in PT boys compared to FT AGA ($p < 0.001$). At 30

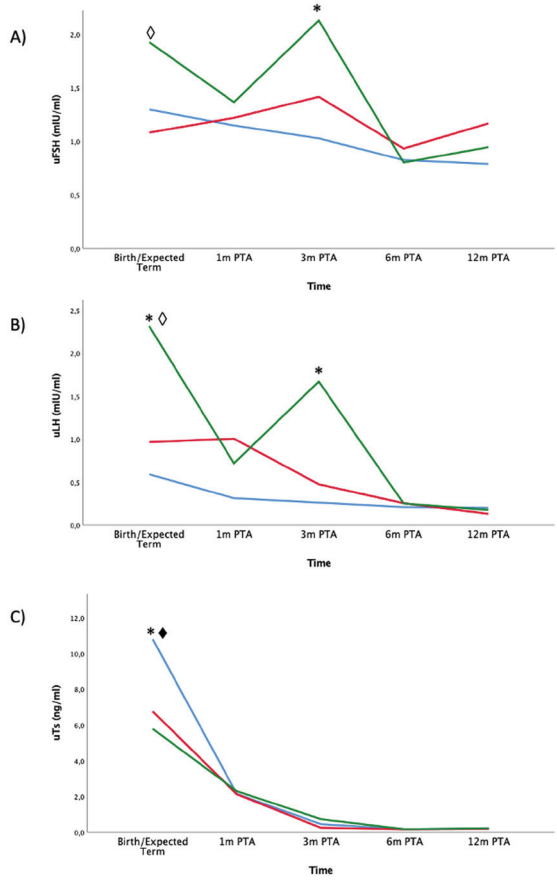

Figure 2. Trend of hormone levels from birth (expected term of GA for PT boys) until 12 months of PTA. Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green). Urine samples were assessed for uFSH (A), uLH (B), uTs (C). Differences statistically significant are indicated with this legend: *FT AGA≠PT, ♦FT AGA≠FT SGA, ◊FT SGA≠PT

uFSH: urinary follicle stimulating hormone, GA: gestational age, PT: preterm, PTA: post-term age, AGA: appropriate for GA, SGA: small for gestational age, HC: head circumference, uLH: urinary luteinizing hormone, uTs: urinary testosterone

days PT male neonates continue to have higher levels of uLH, compared to both FT AGA and FT SGA ($p < 0.001$) and PT babies also had higher uTs levels in comparison with FT AGA boys ($p < 0.05$).

At 90 days of life PT boys still had higher levels of uTs, in comparison with both FT AGA and SGA male babies ($p = 0.015$ and $p < 0.05$, respectively) as shown in Figure 5.

Correlation Analyses

To test the hypothesis that minipuberty correlates with external genitalia development and linear growth, a correlation analysis was performed between uTs levels and auxological parameters (recumbent length and penile length), using the calendar age.

No significant differences were found between uTs and linear growth within the first three months. However, a significant correlation was found between uTs and penile growth (rho = 0.412, $p = 0.04$).

Discussion

The development of highly sensitive immunofluorometric and chemiluminometric assays for measurement of gonadotropin levels in clinical research studies highlighted

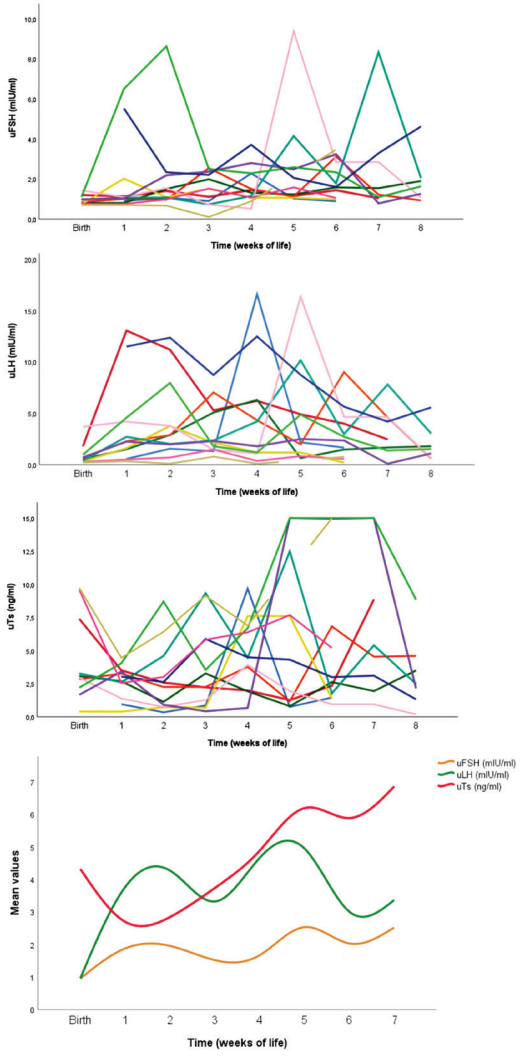

Figure 3. Trends of hormone levels during first weeks of life among PT babies. First three graphs show individual pattern of each premature boy using a different colour for each subject. The graph at the bottom of this figure shows the mean trend of urinary hormone levels of all PT boys

uFSH: urinary follicle stimulating hormone, uLH: urinary luteinizing hormone, uTs: urinary testosterone, PT: preterm

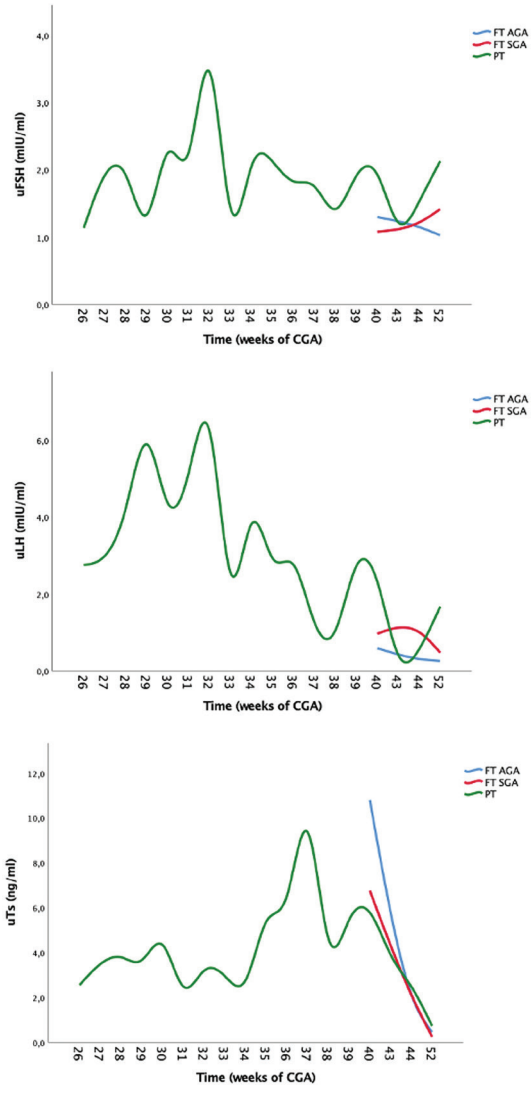

Figure 4. Effects of preterm birth on levels of uFSH, uLH and uTs. Trend of preterm male neonates is compared with full-term neonates until 52 weeks of CGA

uFSH: urinary follicle stimulating hormone, AGA: appropriate for gestational age, SGA: small for gestational age, uLH: urinary luteinizing hormone, uTs: urinary testosterone

a strong correlation between serum and urine samples (37,38,39). Despite this, there are still no validated reference values for uGns in children according to sex, age and pubertal stage that allow their use in routine clinical settings. However, all studies using urine samples to investigate the HPG axis agree on their use in clinical research settings (18,40,41,42).

At birth uGns are low with a subsequent gradual increase and a peak between one week and three months, with uLH levels predominant over uFSHs. Our longitudinal study in male infants allowed the identification of temporal relationships in hormonal levels and their effects on catch-up growth and external genitalia development during the first months. Moreover, splitting neonates into three categories allowed us to better elucidate the influence of birthweight and prematurity on this post-natal HPG axis activation.

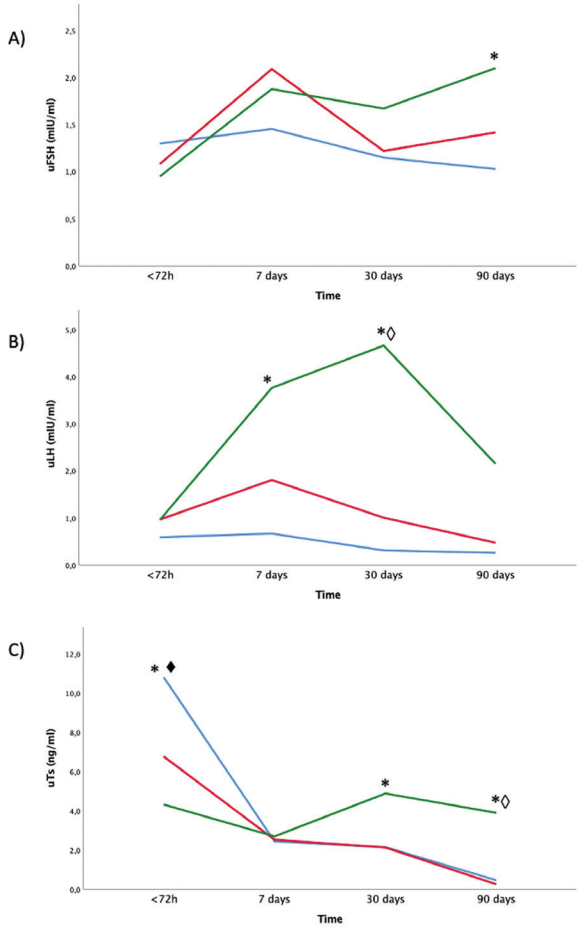

Figure 5. Longitudinal assessment of hormone levels from birth to 3 months of life using calendar age. Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green). Urine samples were assessed for uFSH (A), uLH (B), uTs (C). Differences statistically significant are indicated with this legend: *FT AGA≠PT, ♦FT AGA≠FT SGA, ◊FT SGA≠PT

uFSH: urinary follicle stimulating hormone, uLH: urinary luteinizing hormone, uTs: urinary testosterone, PT: preterm, AGA: appropriate for gestational age, SGA: small for gestational age, HC: head circumference, FT: full-term

The influence of prematurity on sexual hormone levels has been investigated by few studies, mainly cross-sectional and with slightly different findings (30,31,32). Only one study with a longitudinal design (7) found higher uLH and uT levels in PT boys, as well as an increase in T levels in all neonates with a peak at one month and a positive correlation with penile growth. Even the impact of being SGA on the postnatal HPG axis activation is still not elucidate (26,27,29,43). Data are not univocal and the definition itself of a SGA neonate embraces multiple possible criteria (44,45).

Our results are in line with the longitudinal study by Kuiri-Hänninen et al (7). PT boys have higher uLH and uTs levels from one week to three months of life, even if this difference is not always significant. uLH levels at seven days and one month were also higher in FT SGA neonates than in FT AGA boys. uTs levels were higher in PT boys with a postnatal rise of T at around one month in all neonates. uFSH has a higher peak at one week in FT SGA neonates but a subsequent decline towards one month of age. In contrast, uFSH in PT babies peaked later but lasted longer with higher levels at three months in comparison with FT AGA boys. Therefore, PT birth seems to have a great influence both on magnitude and duration of this postnatal HPG axis activation.

However, when comparing neonates depending on PTA, it is interesting to note that differences between FT and PT boys gradually reduced with still higher uGns in PT at the expected term of gestation but no differences at 6- and 12-months PTA.

These findings support the suggestion that the postnatal HPG axis activation is developmentally regulated (1). Postnatal pituitary activation begins with a similar timing in PT and FT neonates but persists longer and higher in PT babies. PT boys thus probably experience a maturation of the hypothalamic feedback mechanisms as term approaches.

Postnatal HPG axis activation has been suggested to also play a role in completing the development of external genitalia (18,40,41). We found a significant correlation between uTs levels and penile growth. However, even if PT boys displayed higher levels of uTs, penile growth of PT boys was not significantly different from FT AGA and FT SGA male neonates, in contrast with previous studies (7,11). Penile length of PT boys showed a catch-up growth during the first months of life leading to no significant differences at 3-6 months of age. Moreover, T surge and testicular descent were strongly related in PT infants.

Coming to the effects of T levels on catch-up growth and in contrast with previous studies (18), no significant correlation between uTs and growth velocity was found, but the recumbent length at 6-months PTA showed no differences between groups, suggesting a catch-up growth of FT SGA and PT boys.

The strength of our study is related to the age of our population. First, we performed a longitudinal analysis with serial samples and a between-group comparison that allows a better analysis of the influence of birth weight and prematurity on the HPG axis. As far as we understand, the present study is the only one to investigate the HPG axis in extremely and moderately PT babies. In parallel, we did not include neonates with a weight between the 4th and 9th percentile, so as not to overlap with other studies and to better appreciate the influence of low birth weight on this axis.

Study Limitations

Some limitations need to be highlighted. First, the sample size was limited. Recruitment of PT babies can be challenging because of their clinical conditions and their lower numbers. Concurrently, the enrolment of healthy infants in a prospective study until 12-months of age can be difficult for families' compliance. We did not perform a correlation analysis with serum samples, but the use of uGns in research studies have been largely developed, making us confident that our results can reflect the pattern of serum gonadotropins and this design also probably improved family compliance. However, it is important to underline how the methodology of this study could have been empowered by the comparison between uGns and serum gonadotrophins and salivary T, especially in PT babies due to the immaturity of the tubular activity.

The need for creatinine correction during the neonatal period is still controversial, especially in neonates (37,38,39,40,46,47). Creatinine clearance is low at birth and the rate is positively correlated with weight, length and post-conceptional age, but negatively correlated with GA (48). This information can explain our lower Ucr values in premature infants and it is probably the reason of the between-groups significant difference. We can hypothesize that the use of uGns not-corrected for creatinine excretion in a comparison between FT and PT neonates was acceptable but this suggestion needs to be interpreted with caution. uTs levels were assessed with a chemiluminescent radioimmunoassay, not with mass spectrometry. This may be relevant, not only for excluding the influence of other androgens, but mainly to detect low levels after the peak of T at 1-3 months. In fact, our results in terms of PTA revealed low to undetectable uTs levels just after three months whereas other studies on serum and urine samples displayed higher levels in male infants until 6-9 months PTA. However the use of an immunoenzymatic method for T detection is in line with other studies that used urine samples (12,18).

Conclusion

Our study provided insight into the postnatal HPG axis activation in FT AGA and SGA boys, as well as in PT infants. The results suggest that minipuberty is increased and prolonged in PT boys in comparison with pairwise FT babies, only when the analysis depends on calendar age, suggesting an ontogenetic regulation. The enhanced activation of the HPG axis translates to a more pronounced androgen secretion with a faster penile growth in PT neonates during the first three months.

However, the results in FT SGA infants are difficult to interpret and need a sample size extension. SGA neonates had the lowest postnatal T levels even if with higher uGns levels and a same trend in penile growth, possibly reflecting a different intrauterine stimulation due to a growth restriction. However, the lack of a postnatal peak of T translated to a slower catch-up growth in FT SGA neonates. In conclusion, minipuberty provides an important window of opportunity for the evaluation of HPG axis functionality before puberty. uGns have been demonstrated to be a valid, practical and non-invasive tool for the purpose; wider acceptance of this method among infants may be clinically beneficial.

The possible short-term and long-term implications of this different postnatal activity in SGA or PT neonates need to be clarified. Further studies with a long-term follow-up are needed with regards to healthy infants but should also take account of birthweight and prematurity.

Acknowledgments

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Ethics

Ethics Committee Approval: The AOU/AUSL Modena Local Ethics Committee approved the protocol (no: 329/17, date: 29.11.2017).

Informed Consent: Informed consent was obtained from all families.

Authorship Contributions

Surgical and Medical Practices: Alessandra Boncompagni, Elisa Pietrella, Erica Passini, Licia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni, Concept: Alessandra Boncompagni, Laura Lucaccioni, Design: Alessandra Boncompagni, Laura Lucaccioni, Data Collection or Processing: Alessandra Boncompagni, Elisa Pietrella, Erica Passini, Chiarina Grisolia, Mara Tagliazucchi, Enrico Tagliafico, Analysis or Interpretation: Alessandra Boncompagni, Chiarina Grisolia, Mara Tagliazucchi, Enrico Tagliafico, Lorenzo Iughetti, Laura Lucaccioni, Literature Search: Alessandra Boncompagni, Licia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni, Writing: Alessandra Boncompagni, Elisa Pietrella, Licia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni.

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References

1. Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr 2014;82:73-80. Epub 2014 Jul 5
2. de Zegher F, Devlieger H, Veldhuis JD. Pulsatile and sexually dimorphic secretion of luteinizing hormone in the human infant on the day of birth. Pediatr Res 1992;32:605-607.
3. Winter JS, Hughes IA, Reyes FI, Faiman C. Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. J Clin Endocrinol Metab 1976;42:679-686.
4. Schmidt H, Schwarz HP. Serum concentrations of LH and FSH in the healthy newborn. Eur J Endocrinol 2000;143:213-215.
5. Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, Gottlieb S, Bergadá C, Campo S, Rey RA. Time course of the serum gonadotropin surge, inhibins, and anti-Müllerian hormone in normal newborn males during the first month of life. J Clin Endocrinol Metab 2006;91:4092-4098. Epub 2006 Jul 18
6. Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metab 1998;83:675-681.
7. Kuiri-Hänninen T, Seuri R, Tyrväinen E, Turpeinen U, Hämäläinen E, Stenman UH, Dunkel L, Sankilampi U. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. J Clin Endocrinol Metab 2011;96:98-105. Epub 2010 Sep 29
8. Forest MG, Cathiard AM, Bertrand JA. Evidence of testicular activity in early infancy. J Clin Endocrinol Metab 1973;37:148-151.
9. Garagorri JM, Rodríguez G, Lario-Elboj AJ, Olivares JL, Lario-Muñoz A, Orden I. Reference levels for 17-hydroxyprogesterone, 11-desoxycortisol, cortisol, testosterone, dehydroepiandrosterone sulfate and androstenedione in infants from birth to six months of age. Eur J Pediatr 2008;167:647-653. Epub 2007 Aug 11
10. Barry JA, Hardiman PJ, Siddiqui MR, Thomas M. Meta-analysis of sex difference in testosterone levels in umbilical cord blood. J Obstet Gynaecol 2011;31:697-702.
11. Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, Damgaard IN, Kai CM, Chellakooty M, Skakkebaek NE, Toppari J, Main KM. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. Eur J Endocrinol 2006;154:125-129.
12. Kuiri-Hänninen T, Haanpää M, Turpeinen U, Hämäläinen E, Dunkel L, Sankilampi U. Transient postnatal secretion of androgen hormones is associated with acne and sebaceous gland hypertrophy in early infancy. J Clin Endocrinol Metab 2013;98:199-206. Epub 2012 Nov 8
13. Janus D, Wojcik M, Tyrawa K, Starzyk J. Transient isolated scrotal hair development in infancy. Clin Pediatr (Phila) 2013;52:628-632. Epub 2013 Mar 11
14. Perry RJ, Farquharson C, Ahmed SF. The role of sex steroids in controlling pubertal growth. Clin Endocrinol (Oxf) 2008;68:4-15. Epub 2007 Jul 20
15. Roche AF, Guo S, Moore WM. Weight and recumbent length from 1 to 12 mo of age: reference data for 1-mo increments. Am J Clin Nutr 1989;49:599-607.
16. Gasser T, Sheehy A, Molinari L, Largo RH. Sex dimorphism in growth. Ann Hum Biol 2000;27:187-197.
17. Becker M, Oehler K, Partsch CJ, Ulmen U, Schmutzler R, Cammann H, Hesse V. Hormonal 'minipuberty' influences the somatic development of boys but not of girls up to the age of 6 years. Clin Endocrinol (Oxf) 2015;83:694-701. Epub 2015 Jul 1
18. Kiviranta P, Kuiri-Hänninen T, Saari A, Lamidi ML, Dunkel L, Sankilampi U. Transient Postnatal Gonadal Activation and Growth Velocity in Infancy. Pediatrics 2016;138:e20153561. Epub 2016 Jun 9
19. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G, Hines M. Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys. Psychol Sci 2009;20:144-148. Epub 2009 Jan 23
20. Lamminmäki A, Hines M, Kuiri-Hänninen T, Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. Horm Behav 2012;61:611-616. Epub 2012 Feb 20
21. Kung KT, Browne WV, Constantinescu M, Noorderhaven RM, Hines M. Early postnatal testosterone predicts sex-related differences in early expressive vocabulary. Psychoneuroendocrinology 2016;68:111-116. Epub 2016 Mar 5
22. Schaadt G, Hesse V, Friederici AD. Sex hormones in early infancy seem to predict aspects of later language development. Brain Lang 2015;141:70-76. Epub 2014 Dec 22
23. Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER. Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab 1993;76:996-1001.
24. Golombok S, Rust J. The measurement of gender role behaviour in pre-school children: a research note. J Child Psychol Psychiatry 1993;34:805-811.
25. Pasterski V, Zucker KJ, Hindmarsh PC, Hughes IA, Acerini C, Spencer D, Neufeld S, Hines M. Increased Cross-Gender Identification Independent of Gender Role Behavior in Girls with Congenital Adrenal Hyperplasia: Results from a Standardized Assessment of 4-

to 11-Year-Old Children. Arch Sex Behav 2015;44:1363-1375. Epub 2014 Sep 20

26. Nagai S, Kawai M, Myowa-Yamakoshi M, Morimoto T, Matsukura T, Heike T. Gonadotropin levels in urine during early postnatal period in small for gestational age preterm male infants with fetal growth restriction. J Perinatol 2017;37:843-847. Epub 2017 Apr 27
27. Ibáñez L, Potau N, Enriquez G, Marcos MV, de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. Hum Reprod 2003;18:1565-1569.
28. Forest MG, de Peretti E, Bertrand J. Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared with that of full-term infants. J Steroid Biochem 1980;12:25-36.
29. Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E. Low birth weight for gestational age and subsequent male gonadal function. J Pediatr 2002;141:376-379.
30. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y. Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. J Clin Endocrinol Metab 1983;56:1327-1331.
31. Greaves RF, Hunt RW, Chiriano AS, Zacharin MR. Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. Pediatrics 2008;121:574-580.
32. Tapanainen J, Koivisto M, Vihko R, Huhtaniemi I. Enhanced activity of the pituitary-gonadal axis in premature human infants. J Clin Endocrinol Metab 1981;52:235-238.
33. Ahmed SF, Achermann JC, Arlt W, Balen A, Conway G, Edwards Z, Elford S, Hughes IA, Izatt L, Krone N, Miles H, O'Toole S, Perry L, Sanders C, Simmonds M, Watt A, Willis D. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015) Clin Endocrinol (Oxf) 2016;84:771-788. Epub 2015 Aug 13
34. Bertino E, Spada E, Occhi L, Coscia A, Giuliani F, Gagliardi L, Gilli G, Bona G, Fabris C, De Curtis M, Milani S. Neonatal anthropometric charts: the Italian neonatal study compared with other European studies. J Pediatr Gastroenterol Nutr 2010;51:353-361.
35. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Onis M de, editor. Geneva: WHO Press; 2006. p. 312. Available from: https://www.who.int/publications/i/item/924154693X
36. Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, Skakkebaek NE, Toppari J. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 2004;363:1264-1269.
37. McNeilly JD, Mason A, Khanna S, Galloway PJ, Ahmed SF. Urinary gonadotrophins: a useful non-invasive marker of activation of the hypothalamic pituitary-gonadal axis. Int J Pediatr Endocrinol 2012;2012:10.
38. Lucaccioni L, McNeilly J, Mason A, Giacomozzi C, Kyriakou A, Shaikh MG, Iughetti L, Ahmed SF. The measurement of urinary gonadotropins for assessment and management of pubertal disorder. Hormones (Athens) 2016;15:377-384.
39. Kolby N, Busch AS, Aksglaede L, Sørensen K, Petersen JH, Andersson AM, Juul A. Nocturnal Urinary Excretion of FSH and LH in Children and Adolescents With Normal and Early Puberty. J Clin Endocrinol Metab 2017;102:3830-3838.
40. Kuiri-Hänninen T, Kallio S, Seuri R, Tyrväinen E, Liakka A, Tapanainen J, Sankilampi U, Dunkel L. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. J Clin Endocrinol Metab 2011;96:3432-3439. Epub 2011 Sep 7
41. Kuiri-Hänninen T, Haanpää M, Turpeinen U, Hämäläinen E, Seuri R, Tyrväinen E, Sankilampi U, Dunkel L. Postnatal ovarian activation has effects in estrogen target tissues in infant girls. J Clin Endocrinol Metab 2013;98:4709-4716. Epub 2013 Nov 11
42. Boncompagni A, McNeilly J, Murtaza M, Lucaccioni L, Iughetti L, Wong SC, Mason A. Clinical utility of urinary gonadotrophins in hypergonadotrophic states as Turner syndrome. J Pediatr Endocrinol Metab 2020;33:1373-1381.
43. Baker TG, Scrimgeour JB. Development of the gonad in normal and anencephalic human fetuses. Reproduction. 1980;60:193-199.
44. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: short stature and beyond. Endocr Rev 2007;28:219-251.
45. 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
46. Demir A, Alfthan H, Stenman UH, Voutilainen R. A clinically useful method for detecting gonadotropins in children: assessment of luteinizing hormone and follicle-stimulating hormone from urine as an alternative to serum by ultrasensitive time-resolved immunofluorometric assays. Pediatr Res 1994;36:221-226.
47. Demir A, Dunkel L, Stenman UH, Voutilainen R. Age-related course of urinary gonadotropins in children. J Clin Endocrinol Metab 1995;80:1457-1460.
48. Mannan MA, Shahidulla M, Salam F, Alam MS, Hossain MA, Hossain M. Postnatal development of renal function in preterm and term neonates. Mymensingh Med J 2012;21:103-108.

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Neurodevelopmental Outcome of Infants with Transient Hypothyroxinemia of Prematurity in a Newborn Intensive Care Unit

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What is already known on this topic?

Transient hypothyroxinemia of prematurity (THOP) is defined as free thyroxine (fT4) level below the reference values despite normal thyroid stimulating hormone level in a preterm infant. THOP occurs in 35-50 % of preterm births and preterm births make up 12 % of all births. The lack of long-term outcome data about infants with THOP presents a challenge for the management of these babies.

What this study adds?

This study provides objective information on neurodevelopmental outcome of the infants with THOP. Levothyroxine replacement was associated with higher rates of steroid use for bronchopulmonary dysplasia, and anti-vascular epithelial growth factor therapy use for retinopathy of prematurity without any effect on long-term neurological outcomes in infants with THOP. Depending on the data provided by this study, clinicians may avoid prophylactic thyroid hormone use in preterm infants with THOP.

Abstract

Objective: The aim of this study was to evaluate neurological development of infants with transient premature hypothyroxinemia (THOP).

Methods: This prospective study included newborns who were born between 28-36 weeks of gestation (GW) and were admitted to the neonatal intensive care unit. Newborns exposed to maternal thyroid disease, or with severe intracranial problems, and congenital anomalies were excluded. Infants with THOP were the study group and those without THOP formed the control group. The study group was subdivided into those receiving levothyroxine replacement (5 µg/kg/day) and those who were untreated. Neonatal demographics, and morbidities, including respiratory distress syndrome, bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP) were evaluated. The Ages and Stages Questionnaire (ASQ) and ASQ:Social-Emotional (ASQ:SE) developmental screening tests were administered to the entire study population at the corrected age of two years.

Results: Seventy infants were included in this study, 40 of whom had THOP. The mean GW was 34.4 ± 3.8 weeks in the study group and 37.2 ± 2.3 weeks in controls (p = 0.69). Mean overall birth weight was 1640 ± 428 g. Levothyroxine replacement was started in 12/40 infants (30 %). The groups were similar in terms of demographic characteristics. Rates of BPD and ROP were higher in the treated group (p = 0.01). ASQ and ASQ:SE results did not differ between groups (p = 0.75), nor did these scores differ between infants with THOP who did or did not receive levothyroxine (p = 0.14).

Conclusion: Although levothyroxine replacement therapy was associated with increased rates of BPD and ROP, this treatment did not appear to improve long-term neurological outcomes in this small group of infants with THOP. Prospective controlled studies with much larger sample sizes are needed to clarify the role of levothyroxine replacement in THOP.

Keywords: Ages and Stages Questionnaire, neurodevelopmental outcome, preterm, transient hypothyroxinemia of prematurity

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Introduction

Transient hypothyroxinemia of prematurity (THOP) is defined as having a lower free thyroxine (fT4) level than age-appropriate reference values, in the context of a normal thyroid stimulating hormone (TSH) level in preterm infants (1). At present, the preterm birth rate is 12% and hypothyroxinemia occurs in 35-50% of all premature infants (2,3).

Hypothyroxinemia seen in preterm infants is usually temporary, and in these cases recovery may require 4-8 weeks. Although it is generally thought that this situation does not affect postnatal development, there are studies suggesting treatment is beneficial in very low birth weight (VLBW) premature infants. However, this issue is still controversial. Several studies examining whether preterm hypothyroxinemia affects development in infancy-early childhood have reported inconsistent results (4). Physiologically lower thyroid hormone levels in preterm newborns has been explained by thyroxine (T4) conversion into inadequate triiodothyronine (T3) and premature cessation of maternal T4 transport, increased thyroid hormone requirement (thermoregulation and muscle function), iodine metabolism dysregulation, lack of the maturity of the hypothalamic-pituitary-thyroid axis, and inadequate response of the thyroid gland to thyrotropin-releasing hormone (TRH) or a combination of these (5,6). In a large-scale study on long-term outcomes, when 398 infants born before the gestational age of 32 weeks and birth weight < 1500 grams were evaluated at the age of 19 years, no negative effect of THOP on intelligence quotient or motor functions was observed (7). In a randomized controlled study, no positive findings were found in the 36-month examinations of infants younger than 28 weeks of gestation who were treated (8). In contrast, another study reported better language skills, motor and cognitive functions in the thyroxine-replacement group (9). It should be kept in mind that thyroxine replacement to correct hypothyroxinemia may also have side effects in premature infants. Circulatory dysfunction was reported following thyroxine treatment in VLBW premature infants (10). Hence the recommendation about thyroid hormone therapy for premature infants with THOP is not clarified yet and it is open to discussion (2). There is no published study in which the neurological development of infants diagnosed with THOP is compared with a control group with a similar gestational week and at the same age. Therefore, the aim of this study was to evaluate the neurological development of infants with THOP compared to a matched control group and to investigate the effects of perinatal and neonatal risk factors on outcomes.

Methods

This prospective study included newborn infants who were born at 28-36 gestational weeks and were hospitalized in the neonatal intensive care unit between January 2020 and April 2021. Newborn infants with THOP constituted the study group while infants with similar demographic characteristics, but without THOP, formed the control group. Levothyroxine was started in a sub-group of the infants with THOP using the random number assignment method following informed consent being obtained from the parents. In cases when the parents did not give informed consent for randomization, the infants were assigned to the group without replacement, if the parents approved. Since this simple randomization method was used, group numbers were not the same. Levothyroxine was started for the replacement group at a dose of 5 µg/kg/day. Due to the unavailability of the liquid formulation at the study center, an oral suspension with a concentration of 25 mcg/mL is prepared using 100 mcg tablets by the pharmacy. Twenty-five 100 mcg LT4 tablets were crushed in a mortar to obtain a fine powder. A small amount of glycerol was added to the powder and thoroughly mixed to create a uniform suspension. This mixture was then transferred to a 100 mL calibrated amber bottle. The mortar was rinsed with 10 mL of glycerol, which was then poured into the bottle. This process was repeated until all 40 mL of glycerol was used. Finally, water was added to bring the total volume up to 100 mL. This suspension was well-shaken before use and refrigerated for a week. The necessary dose is adjusted weekly (at the same day of the week) according to the actual weight of the newborn. Besides, TFTs were checked weekly until the normal ranges were observed, then monthly measurements continued during replacement. Once started, levothyroxine replacement was planned to continue until two years of age.

Initial thyroid function tests (TFT) were taken on the 10-20th days of life. In addition to the national screening program at least two measurements performed at 10th, 17th days and more if necesseray. TFTs, including free thyroxine (fT4; ng/dL) and thyroid stimulating hormone (TSH; mIU/L) were performed by electrochemiluminescence immunoassay (ECLIA, Cobas e602, Roche Diagnostics, Basel, Switzerland).

Newborns with maternal thyroid disease, severe intracranial issues, chromosomal and congenital anomalies, or infants older than 36 gestational weeks were not included in the study. Normal ranges of TFTs were evaluated according to postnatal age and gestational week (2). THOP was defined as transient low fT4 levels without elevation in TSH levels (11). A TSH level > 10 mIU/L detected after the first week

was considered elevated/high; fT4 levels were considered low if < 1.3 ng/dL in 31-36 gestational week-old infants and < 0.5 ng/dL in 25-30 gestational week-old infants (12). In addition to THOP, demographic and clinical data including antenatal steroid therapy, inotropic support and the presence of respiratory distress syndrome (RDS), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA) and maternal disease characteristics were recorded in all infants.

Validated Turkish versions of the 'ASQ' and 'ASQ:SE' developmental screening tests were administered to the entire study population at the corrected age of two years.

The Use of the 'Ages And Stages Questionnaire' (ASQ) and 'ASQ:Social-Emotional' (ASQ:SE) Developmental Screening Tests in the Evaluation of Neurological Development

Neurological development is generally evaluated by screening tools and expert assessment, which may also include information from parents (13,14,15).

ASQ and ASQ:SE Inventories

The 'ASQ' is a screening tool for assessing the development of infants and preschoolers in the areas of communication, fine motor, gross motor, problem-solving and personal-social development, and 'ASQ:SE' is a screening tool for social-emotional development, based on information given by parents (16). Küçüker et al. (16) evaluated 608 children with the Turkish version of 'ASQ:SE'. When all months were analyzed, the sensitivity of ASQ was 83.7%, specificity was 89.9%, test-retest reliability was 87%, and inter-rater reliability was 83.6%.

Ethics

This study was approved by Ordu University Training and Research Hospital Local Ethics Committee (file number: 2021/249, date: 19.11.2021). Informed consent for participation was obtained from the parents. This study is registered in ClinicalTrials.gov with the number of NCT05901623.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS), version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Clinical data are presented as means ± standard deviation for parametric tests and categorical data displayed as mean rank for nonparametric tests. The Kolmogorov-Smirnov test was used to check the distribution of the variables. Comparisons were performed by the use of the t-test for normally distributed variables, or the Mann-Whitney U test in the case of non-parametric distribution. The chi-square test was used for qualitative data analysis. Statistical significance was accepted in case of a probability (p) value < 0.05. The sample size calculation using a one-sided McNemar's test has determined that a total of 42 participants, with at least 21 in each group, is sufficient to detect a clinically significant difference between the groups, with 80% power and a 5% level of significance.

Results

A total of 70 newborn infants were included, of whom 40 had THOP and the remainder were assigned to the control group. Of the infants with THOP, 55% (n = 22) were male. Sex mix in the control group was 63.3% (n = 19) male and 36.7% (n = 11) female. A comparison of demographic characteristics between the infants with and without THOP is shown in Table 1. There was no significant difference between the gestational weeks of the two groups ($p = 0.69$), nor was there a difference in birth weight ($p = 0.87$) or birth length. There was a significant difference in delivery mode, with infants in the THOP group being more likely to have been delivered by cesarean section ($p < 0.001$). The mean age at which children in the THOP and control groups were tested with the ASQ was 14.03 ± 1.67 and 13.73 ± 1.39 months ($p = 0.43$). The corrected age at the time of ASQ test was 20.7 ± 7.68 months in infants with THOP and 21.8 ± 7.95 months in control group ($p = 0.56$).

Neonatal morbidities, including RDS, BPD, IVH, hsPDA, ROP, sepsis, and duration of mechanical ventilation, steroid use for BPD or anti-vascular endothelial growth factor (anti-VEGF) use for ROP did not differ between the THOP and control groups (Table 2).

The day of first TFT was 14.03 ± 1.67 days in infants with THOP and 13.73 ± 1.39 days in control group ($p = 0.43$). The first value for fT4 was significantly lower in infants with THOP (5.02 ± 2.2 ng/dL) than the control group (14.99 ± 1.29 ng/dL), as expected ($p < 0.001$). Furthermore, the first value of TSH was significantly higher in infants with THOP (4.85 ± 2.2 mIU/L) than the control group (2.34 ± 0.66 mIU/L) ($p < 0.001$). When started, levothyroxine replacement was continued to two years of age. Only one infant stopped replacement before the age of two years because of TSH suppression.

No difference was found in the comparison of the 'ASQ and ASQ:SE' results of the THOP and control groups (Table 3).

Demographic characteristics were similar between the infants with THOP who did and did not receive levothyroxine replacement, except there was a significantly higher rate of antenatal steroid use in infants receiving levothyroxine compared to the infants who did not (Table 4). Testing time for initial fT4 and TSH level, and corrected age at ASQ evaluation time did not differ between the infants with and without L-T4 replacement.

There was no difference in rates of RDS, invasive mechanical ventilator support, hsPDA, IVH, sepsis, and blood transfusions between the infants with THOP who did or did not receive levothyroxine replacement (Table 5). However, the duration of non-invasive mechanical ventilation, and the rates of BPD, steroid use for BPD, ROP, and anti-VEGF use for ROP rates were significantly higher in infants with THOP who received levothyroxine. Furthermore, 'ASQ' and 'ASQ:SE' results did not differ between those infants

Table 1. Comparison of demographic characteristics between the infants with and without hypothyroxinemia

		Hypothyroxinemia (-) (n = 30)			Hypothyroxinemia (+) (n = 40)			p
		Mean ± SD/n-%		Mean rank	Mean ± SD/n-%		Mean rank	
Maternal age		29.4 ± 4.1			28.1 ± 4.2			0.74
Sex	Female	11	36.70%		18	45%		0.63
	Male	19	63.30%		22	55%		
Delivery mode	NSV	2	66.70%		1	33.30%		< 0.001
	C/S	28	41.80%		39	58.20%		
Birth weight		1394.5 ± 501.6			1359.5 ± 498.1			0.87
Gestational age		37.2 ± 2.3		36.6	34.4 ± 3.8		34.7	0.69
APGAR 1st min				36.93			34.42	0.6
APGAR 5th min				35.3			35.65	0.94
Antenatal steroid		6	20.00%		7	17.50%		0.79
SGA		3	10.00%		5	12.50%		0.94
PPROM		4	13.30%		7	17.50%		0.83
Chorioamnionitis		3	10.00%		4	10.00%		1
Urinary tract infection		4	13.33%		3	8.00%		0.54
GDM		1			0			N/A
Preeclampsia		3	10.00%		4	10.00%		1

Mann-Whitney U test, t-test, chi-square test.
SD: standard deviation, SGA: small for gestational age, PPROM: preterm premature rupture of membranes, N/A: not applicable, C/S: cesarean section, GDM: gestational diabetes mellitus

Table 2. Comparison of neonatal morbidities between infants with and without hypothyroxinemia

	Hypothyroxinemia (-) (n = 30)			Hypothyroxinemia (+) (n = 40)			p
	Mean ± SD/n-%		Mean rank	Mean ± SD/n-%		Mean rank	
RDS	24	80%		34	85%		0.89
IMV (hours)			36.7			34.6	0.67
NIMV (hours)	83.30 ± 51.03		36.85	76.3 ± 38.32		34.49	0.54
hsPDA	5	17%		8	20%		0.77
IVH (> grade 2)	9	30%		15	37.5%		0.8
Sepsis	10	33.3%		12	30%		0.64
ES tx	18	60%		25	62.5%		0.51
BPD	4	12%		7	28.00%		0.75
Steroid for BPD	4	12%		7	28%		0.75
ROP	10	33.30%		12	30%		0.64
Anti-VEGF for ROP	4	12%		7	28%		0.75

Mann-Whitney U test, t-test, chi-square test.
RDS: respiratory distress syndrome, SD: standard deviation, IVH: intraventricular hemorrhage, BPD: bronchopulmonary dysplasia, ROP: retinopathy of prematurity IMV: invasive mechanical ventilation, NIMV: non-invasive mechanical ventilation, hsPDA: hemodynamycally significant patent ductus arteriosus, ES: erytrocyte suspension, tx: transfusion, VEGF: vascular endothelial growth factor

with THOP who received and did not receive levothyroxine replacement (Table 6).

Discussion

This study illustrated that the replacement of levothyroxine did not lead to improved long-term neurological outcomes in the infants with THOP in comparision an age-matched control group.

Risk factors for THOP include infants with low gestational weeks, preeclampsia, intrauterine growth restriction, perinatal asphyxia, NEC, PDA, RDS, IVH, BPD, mechanical ventilation, and receiving medications such as dopamine or dexamethasone (17,18,19,20,21). Various studies have

Table 3. Comparison of ASQ testing results between the infants with and without hypothyroxinemia

		Hypothyroxinemia (-) (n = 30)		Hypothyroxinemia (+) (n = 40)		p
		n- %		n- %		
ASQ score	Normal	5	16.70 %	6	15 %	0.75
	Risk	17	56.70 %	22	55 %	
Follow-up required		8	26.7 %	12	30	
ASQ emotional	Normal	13	43.30 %	15	37.50 %	0.4
	Risk	17	56.70 %	25	62.50 %	
Communication	Normal	16	53.30 %	21	52.50 %	0.57
	Risk	14	46.60 %	19	47.50 %	
Gross motor	Normal	29	96.70 %	38	95 %	0.61
	Risk	1	3.30 %	2	55	
Fine motor	Normal	5	16.70 %	8	20 %	0.49
	Risk	25	83.30 %	32	80 %	
Problem-solving	Normal	16	53.30 %	17	42.50 %	0.26
	Risk	14	46.60 %	23	57.50 %	
Personal-social	Normal	16	53.30 %	18	45 %	0.33
Risk		14	46.60 %	22	55 %	

Chi-square test.
ASQ: Ages and Stages Questionnaire

Table 4. Comparison of characteristics between the infants diagnosed with THOP with and without levothyroxine replacement

		No levothyroxine (n = 28)			Levothyroxine (n = 12)			p
		Mean ± SD/n- %		Mean rank	Mean ± SD/n- %		Mean rank	
Maternal age		28.07 ± 4.6			28.25 ± 3.4			0.89
Sex	Female	15	53.57 %		5	41.67 %		0.53
	Male	13	46.43 %		7	58.33 %		
Delivery mode	NSV	6	21.43 %		2	16.67 %		0.8
	C/S	22	78.57 %		10	83.33 %		
Birth weight		1393.57 ± 468.74			1280 ± 574.77			0.52
Gestational age		30.18 ± 2.9			29.42 ± 3.99			0.32
APGAR 1st min				20.04			21.58	0.69
APGAR 5th min				20.96			19.42	0.67
Antenatal steroid		2	7.14 %		5	41.67 %		**0.02**
SGA		3	10.70 %		2	16.70 %		0.46
First TFT (postnatal day of life)		14.04 ± 1.7			14.75 ± 1.8			0.09
First value of fT4 (ng/dL)		5.02 ± 2.2			4.70 ± 1.7			0.66
First value of TSH (mIU/L)		4.74 ± 2.03			5.10 ± 2.63			0.64
Corrected age at ASQ testing (months)		20.36 ± 7.7			21.50 ± 7.88			0.68

Mann-Whitney U test, t-test, chi-square test.
SGA: small for gestational age, THOP: transient hypothyroxinemia of prematurity, ASQ: Ages and Stages Questionnaire, fT4: free thyroxine, TFT: thyroid function test, TSH: thyroid stimulating hormone, SD: standard deviation

shown that some medications, as well as non-thyroidal diseases such as RDS, sepsis, IVH, PDA, and NEC, are associated with THOP, and the degree of hypothyroxinemia correlates with the severity of such diseases (22,23,24). Furthermore, thyroid functions may be repressed due to commonly used medications including dopamine and dexamethasone, as well as by the presence of RDS, sepsis, NEC, PDA, malnutrition, and chorioamnionitis (5,22,25,26). Serum thyroid hormone levels are partially mediated by acute inflammatory cytokines in different pathologies such as RDS, PDA, sepsis, IVH, and NEC (27). In recent years, studies have shown that the suppression of thyroid functions decreases along with the decrease in the severity of RDS due to the improvement of antenatal care, particularly the rational use of antenatal steroids and the timely surfactant application (27).

In the present study and in contrast to previous studies, RDS, PDA, sepsis, need for inotropic medications, IVH, and blood transfusion were not identified as factors associated with THOP. The reason for these different results in our study may be that surfactants and antenatal steroids have become widespread in recent years, resulting in less need

Table 5. Comparison of neonatal morbidities between the infants with and without levothyroxine replacement

	No levothyroxine (n = 28)			Levothyroxine (n = 12)			p
	Mean ± SD/n-%		Mean rank	Mean ± SD/n-%		Mean rank	
RDS	24	85.70%		10	83.30%		0.6
IMV (hours)	87.64 ± 66.88		19.27	185.5 ± 44.78		23.38	0.06
NIMV (hours)	66.12 ± 30.01			100.5 ± 45.74			**0.03**
hsPDA	5	17.86%		3	25.00%		0.39
IVH (> grade 2)	21	75.00%		9	75.00%		0.51
Sepsis	13	46.43%		6	50.00%		0.89
ES tx	16	57.14%		9	75.00%		0.48
BPD	5	17.86%		7	58.33%		**0.01**
Steroid for BPD	2	7.14%		5	41.67%		**0.02**
ROP	5	17.86%		7	58.33%		**0.01**
Anti-VEGF for ROP	2	7.14%		5	41.67%		**0.02**

Mann-Whitney U test, t-test, chi-square test.
RDS: respiratory distress syndrome, SD: standard deviation, IVH: intraventricular hemorrhage, BPD: bronchopulmonary dysplasia, ROP: retinopathy of prematurity, IMV: invasive mechanical ventilation, NIMV: non-invasive mechanical ventilation, hsPDA: hemodynamycally significant patent ductus arteriosus, ES: erytrocyte suspension, tx: transfusion, VEGF: vascular endothelial growth factor

Table 6. Comparison of ASQ testing results between the infants diagnsed with THOP who did or did not receive levothyroxine

		No levothyroxine (n = 28)		Levothyroxine (n = 12)		p
		n-%		n-%		
ASQ score	Normal	4	14.30%	2	16.70%	0.14
	Risk	13	46.40%	9	75.00%	
Follow-up required		11	39.30%	1	8.30%	
Emotional	Normal	12	43.00%	3	25.00%	0.48
	Risk	16	47.00%	9	75.00%	
Communication	Normal	16	57.10%	5	41.70%	0.49
	Risk	12	42.90%	7	58.30%	
Gross motor	Normal	27	96.40%	11	91.70%	0.52
	Risk	1	3.60%	1	8.30%	
Fine motor	Normal	6	21.40%	2	16.70%	0.55
	Risk	22	78.60%	10	83.30%	
Problem-solving	Normal	14	50.00%	3	25.00%	0.18
	Risk	14	50.00%	9	75.00%	
Personal-social	Normal	15	53.60%	3	25.00%	0.17
Risk		13	46.40%	9	75.00%	

Chi-square test.
ASQ: Ages and Stages Questionnaire, THOP: transient hypothyroxinemia of prematurity

for treatments and their temporary effects, and a decrease in disease severity in NICU. Tan et al. (28) found no relationship between PDA, IVH, antenatal steroid use, ROP, APGAR scores, sepsis, and THOP.

Premature and/or low birth weight infants may exhibit clinical features attributable to thyroid dysfunction, such as temperature imbalance, immature lung function, and inadequate surfactant reserves, as well as apnea, bradycardia, severe intestinal motility and slowed feeding, edema, and tardy growth and development. Several observational studies have demonstrated an association between low serum T4 levels and these clinical presentations (14,29).

In a study with preterm infants <1500 g, no significant differences were found between infants with and without hypothyroxinemia in terms of neurodevelopmental, visual, or hearing impairment at five years of age (28). In a double-blind, randomized, placebo-controlled study in preterm infants less than 28 GW from the UK, the thyroid hormone-treated group had significantly higher scores in language and cognitive domains and better motor skills as assessed by the Bayley III Mental and Psychomotor Developmental Indexes at 42 months (30). Randomized, placebo-controlled studies revealed complex results with some potential benefit of levothyroxine therapy in infants <28 weeks, but potential harm in infants older than 28 weeks (31). TFTs of premature infants can be affected by medications, free fatty acids, protein concentration, and use of furosemide and heparin (32). THOP is an important consideration because it occurs during a critical period for brain development, despite having a self-limiting course. Long-term studies found associations between THOP and mental development abnormalities in preterm infants, particularly reaching milestones later, lower scores on cognitive tests, increased school failure, and cerebral palsy risk (23,33). Yamamoto et al. (33) showed that the results of infants with THOP born under 30 GW to the TRH stimulation test was similar to that of infants with normal thyroid functions, suggesting that the hypothalamohypophyseal thyroid axis is functioning normally in infants with THOP. Depending on these results L-T4 replacement is thought to be non-essential in THOP. Furthermore, L-thyroxine replacement to preterm infants was thought to be safe, as in term infants. However, a case of circulatory disorder related to levothyroxine replacement was reported in a VLBW infant from Japan (34,35,36). Levothyroxine use is reported to increase the relative adrenal insufficiency of prematurity (37). Adrenal insufficiency is more evident if infants with panhypopituitarism receive levothyroxine replacement without glucocorticoids (37). Thus thyroid hormones enhance cortisol turn-over as well as the need for glucocorticoids. Also of concern is a probable increased risk of NEC due to the L-T4 administration (38). Besides, theoretical clinical risks may exist, such as unexplained tachycardia or arrhythmias, tremors, weight loss or hyperpyrexia, and neurobehavioral abnormalities due to excess or unnecessary use of thyroid hormone (39). Thyroid hormones are powerful biological triggers that can cause unexpected effects when given unnecessarily. Hence, the existing evidence suggest that L-T4 replacement brings no clinical benefit but can even harm infants with THOP (40).

One of the most important advantages of the development assessment tools the 'ASQ' with the 'ASQ:SE', which are filled directly by the parents, is that they benefit from the rich experience of the parents about their child and provide the opportunity to continuously evaluate the development of the child at certain time intervals. Research results support that parents can provide accurate information about their children's development regardless of socioeconomic level, region of residence, or mental health (14).

The high agreement between mothers' or fathers' evaluations of their children's developmental skills and expert evaluations and the low cost of evaluation triggered the development of evaluation tools filled by mothers or fathers (13). In 2006, the Council of Children with Disabilities outlined tools that can be safely used as a general developmental screening test (15).

In the present study, no association was found between the 'ASQ' with the 'ASQ:SE' subparameters of THOP in the first two years of life and adverse neurodevelopmental outcomes. For these studies that revealed associations between thyroid hormone abnormalities and adverse neurodevelopmental outcomes in preterm infants, we hypothesized that thyroid status may only be a confounder, that reflects the severity of disease or co-morbidities, such as IVH, which were actual risk factors associated with neurodevelopmental disorder. The study of Fisher (41) supported this hypothesis by showing low T4 levels and low T3 levels in preterm infants, were the result of non-thyroidal diseases and mirrored the disease severity in those infants. It has been suggested that decreased thyroid hormone synthesis, protein catabolism, and oxygen consumption in preterm infants may be a potentially beneficial adaptive response to the disease state. Furthermore, studies by Williams et al. (42) and Carrascosa et al. (43) examining the effect of RDS, IVH, NEC, and sepsis on thyroid function supported this phenomenon. Interestingly, the present study found that anti-VEGF use for ROP, and steroid use for BPD rates were higher in THOP group that received levothyroxine. Further much larger studies are required to elucidate the association with levothyroxine replacement and higher rates of steroid use

for BPD, and anti-VEGF use for ROP without affecting long-term neurological outcomes in infants with THOP. However, this association may be because those infants were 'sicker' or had more advanced stage of preterm morbidities than the others, then it would not be surprising for them to require further therapy for BPD and/or ROP too. Hence, these novel results can also be considered supportive findings. Ultimately, these data suggest that THOP may be an epiphenomenon of non-thyroidal disease but this does not appear to lead to long-term adverse outcomes in infants. Therefore, we believe that better clinical management of risky conditions in preterm infants may reduce the prevalence of THOP and the possible negative consequences that may develop thereafter, rather than routinely treating THOP with levothyroxine replacement (44).

Study Limitations

Although the statistical analysis indicated that the number of participants is sufficient to report, it is evident that the strait number of participants poses a limitation for this study. The other limitations of our study is that we did not question parental intelligence, home environment, breastfeeding or formula feeding, and post-discharge medication use histories.

Conclusion

This study demonstrated that levothyroxine replacement did not improve long-term neurological outcomes in this population of infants with THOP. However, given the limitations of this study, well-designed, much larger, prospective, controlled studies are needed to clarify the role of levothyroxine replacement in THOP.

Ethics

Ethics Committee Approval: This study was approved by Ordu University Training and Research Hospital Local Ethics Committee (file number: 2021/249, date: 19.11.2021).

Informed Consent: Informed consent for participation was obtained from the parents.

Authorship Contributions

Surgical and Medical Practices: Erhan Aygün, Seda Yılmaz Semerci, Adviye Çakıl Sağlık, Emine Yurdakul Ertürk, Concept: Erhan Aygün, Seda Yılmaz Semerci, Design: Erhan Aygün, Seda Yılmaz Semerci, Data Collection or Processing: Erhan Aygün, Seda Yılmaz Semerci, Adviye Çakıl Sağlık, Emine Yurdakul Ertürk, Analysis or Interpretation: Erhan Aygün, Seda Yılmaz Semerci, Literature Search: Erhan Aygün, Seda Yılmaz Semerci, Adviye Çakıl Sağlık, Emine Yurdakul Ertürk, Writing: Erhan Aygün, Seda Yılmaz Semerci.

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References

1. Eerdekens A, Langouche L, Van den Berghe G, Verhaeghe J, Naulaers G, Vanhole C. Review shows that thyroid hormone substitution could benefit transient hypothyroxinaemia of prematurity but treatment strategies need to be clarified. Acta Paediatr 2019;108:792-805. Epub 2019 Jan 4
2. Özon A, Tekin N, Şıklar Z, Gülcan H, Kara C, Taştekin A, Demir K, Koç E, Evliyaoğlu O, Kurtoğlu S. Neonatal effects of thyroid diseases in pregnancy and approach to the infant with increased TSH: Turkish Neonatal and Pediatric Endocrinology and Diabetes Societies consensus report. Turk Pediatri Ars 2019;53(Suppl 1):209-223.
3. Kurtoğlu S, Özdemir A. Fetal neonatal hyperthyroidism: diagnostic and therapeutic approachment. Turk Pediatri Ars 2017;52:1-9.
4. Kadivar M, Parsaei R, Setoudeh A. The relationship between thyroxine level and short term clinical outcome among sick newborn infants. Acta Med Iran 2011;49:93-97.
5. Williams F, Hume R. The measurement, definition, aetiology and clinical consequences of neonatal transient hypothyroxinaemia. Ann Clin Biochem 2011;48:7-22.
6. La Gamma EF, Korzeniewski SJ, Ballabh P, Paneth N. Transient Hypothyroxinemia of Prematurity. Neoreviews 2016;17:394-402.
7. Hollanders JJ, Israëls J, van der Pal SM, Verkerk PH, Rotteveel J, Finken MJ; Dutch POPS-19 Collaborative Study Group. No Association Between Transient Hypothyroxinemia of Prematurity and Neurodevelopmental Outcome in Young Adulthood. J Clin Endocrinol Metab 2015;100:4648-4653. Epub 2015 Oct 19
8. van Wassenaer-Leemhuis A, Ares S, Golombek S, Kok J, Paneth N, Kase J, LaGamma EF. Thyroid hormone supplementation in preterm infants born before 28 weeks gestational age and neurodevelopmental outcome at age 36 months. Thyroid 2014;24:1162-1169. Epub 2014 May 21
9. van Wassenaer AG, Kok JH, de Vijlder JJ, Briët JM, Smit BJ, Tamminga P, van Baar A, Dekker FW, Vulsma T. Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. N Engl J Med 1997;336:21-26.
10. Yagasaki H, Kobayashi K, Nemoto A, Naito A, Sugita K, Ohyama K. Late-onset circulatory dysfunction after thyroid hormone treatment in an extremely low birth weight infant. J Pediatr Endocrinol Metab 2010;23:153-158.
11. Fisher DA. Thyroid system immaturities in very low birth weight premature infants. Semin Perinatol 2008;32:387-397.
12. Adams LM, Emery JR, Clark SJ, Carlton EI, Nelson JC. Reference ranges for newer thyroid function tests in premature infants. J Pediatr 1995;126:122-127.
13. Küçüker S, Kapçı EG, Uslu R. Erken Gelişim Evreleri Envanteri, 2015.
14. Mercado M, Szymonowicz W, Yu VY, Gold H. Symptomatic hypothyroxinemia with normal TSH levels in preterm infants. Clin Pediatr (Phila) 1987;26:343-346.
15. Council on Children With Disabilities; Section on Developmental Behavioral Pediatrics; Bright Futures Steering Committee; Medical Home Initiatives for Children With Special Needs Project Advisory Committee. Identifying infants and young children with developmental

disorders in the medical home: an algorithm for developmental surveillance and screening. Pediatrics 2006;118:405-420.

16. Küçüker S, Kapci EG, Uslu R. Evaluation of the Turkish version of the "Ages and Stages Questionnaires: Social-emotional" in identifying children with social-emotional problems. Infants Young Child 2011;24:207-220.
17. Kantor Herring MJ, Leef KH, Locke RG, Stefano JL, Bartoshesky L, Paul DA. Are perinatal risk factors helpful in predicting and optimizing treatment strategies for transient hypothyroxinemia in very-low-birth-weight infants? Am J Perinatol 2003;20:333-339.
18. Filippi L, Cecchi A, Tronchin M, Dani C, Pezzati M, Seminara S, Gasperini S, Zammarchi E, Rubaltelli FF. Dopamine infusion and hypothyroxinaemia in very low birth weight preterm infants. Eur J Pediatr 2004;163:7-13. Epub 2003 Nov 25
19. Tan LO, Tan MG, Poon WB. Correction: Lack of association between hypothyroxinemia of prematurity and transient thyroid abnormalities with adverse long term neurodevelopmental outcome in very low birth weight infants. PLoS One 2019;14:e0223867.
20. Williams FL, Mires GJ, Barnett C, Ogston SA, van Toor H, Visser TJ, Hume R; Scottish Preterm Thyroid Group. Transient hypothyroxinemia in preterm infants: the role of cord sera thyroid hormone levels adjusted for prenatal and intrapartum factors. J Clin Endocrinol Metab 2005;90:4599-4606. Epub 2005 May 10
21. Woo HC, Lizarda A, Tucker R, Mitchell ML, Vohr B, Oh W, Phornphutkul C. Congenital hypothyroidism with a delayed thyroid-stimulating hormone elevation in very premature infants: incidence and growth and developmental outcomes. J Pediatr 2011;158:538-542. Epub 2011 Jan 13
22. Williams FL, Ogston SA, van Toor H, Visser TJ, Hume R. Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. J Clin Endocrinol Metab 2005;90:5954-5963. Epub 2005 Aug 16
23. Zdraveska N, Kocova M. Thyroid function and dysfunction in preterm infants-Challenges in evaluation, diagnosis and therapy. Clin Endocrinol (Oxf) 2021;95:556-570. Epub 2021 Apr 25
24. Simpson J, Williams FL, Delahunty C, van Toor H, Wu SY, Ogston SA, Visser TJ, Hume R; Scottish Preterm Thyroid Group. Serum thyroid hormones in preterm infants and relationships to indices of severity of intercurrent illness. J Clin Endocrinol Metab 2005;90:1271-1279. Epub 2004 Dec 21
25. van Wassenaer AG, Kok JH, Dekker FW, de Vijlder JJ. Thyroid function in very preterm infants: influences of gestational age and disease. Pediatr Res 1997;42:604-609.
26. Zung A, Bier Palmon R, Golan A, Troitzky M, Eventov-Friedman S, Marom R, Keidar R, Kats N, Almashanu S, Flidel-Rimon O. Risk Factors for the Development of Delayed TSH Elevation in Neonatal Intensive Care Unit Newborns. J Clin Endocrinol Metab 2017;102:3050-3055.
27. Williams FL, Mires GJ, Barnett C, Ogston SA, van Toor H, Visser TJ, Hume R; Scottish Preterm Thyroid Group. Transient hypothyroxinemia in preterm infants: the role of cord sera thyroid hormone levels adjusted for prenatal and intrapartum factors. J Clin Endocrinol Metab 2005;90:4599-4606.
28. Tan LO, Tan MG, Poon WB. Lack of association between hypothyroxinemia of prematurity and transient thyroid abnormalities with adverse long term neurodevelopmental outcome in very low birth weight infants. PLoS One 2019;14:e0222018.
29. Hadeed AJ, Asay LD, Klein AH, Fisher DA. Significance of transient postnatal hypothyroxinemia in premature infants with and without respiratory distress syndrome. Pediatrics 1981;68:494-498.
30. Ng SM, Turner MA, Weindling AM. Neurodevelopmental Outcomes at 42 Months After Thyroxine Supplementation in Infants Below 28 Weeks' Gestation: A Randomized Controlled Trial. Thyroid 2020;30:948-954. Epub 2020 Mar 17
31. LaFranchi SH. Thyroid Function in Preterm/Low Birth Weight Infants: Impact on Diagnosis and Management of Thyroid Dysfunction. Front Endocrinol (Lausanne) 2021;12:666207.
32. Delahunty C, Falconer S, Hume R, Jackson L, Midgley P, Mirfield M, Ogston S, Perra O, Simpson J, Watson J, Willatts P, Williams F; Scottish Preterm Thyroid Group. Levels of neonatal thyroid hormone in preterm infants and neurodevelopmental outcome at 5 1/2 years: millennium cohort study. J Clin Endocrinol Metab 2010;95:4898-4908. Epub 2010 Aug 18
33. Yamamoto A, Kawai M, Iwanaga K, Matsukura T, Niwa F, Hasegawa T, Heike T. Response to thyrotropin-releasing hormone stimulation tests in preterm infants with transient hypothyroxinemia of prematurity. J Perinatol 2015;35:725-728. Epub 2015 Jun 25
34. Kawai M, Kusuda S, Cho K, Horikawa R, Takizawa F, Ono M, Hattori T, Oshiro M. Nationwide surveillance of circulatory collapse associated with levothyroxine administration in very-low-birthweight infants in Japan. Pediatr Int 2012;54:177-181. Epub 2012 Jan 13
35. Okada J, Iwata S, Hirose A, Kanda H, Yoshino M, Maeno Y, Matsuishi T, Iwata O. Levothyroxine replacement therapy and refractory hypotension out of transitional period in preterm infants. Clin Endocrinol (Oxf) 2011;74:354-364.
36. Yagasaki H, Kobayashi K, Nemoto A, Naito A, Sugita K, Ohyama K. Late-onset circulatory dysfunction after thyroid hormone treatment in an extremely low birth weight infant. J Pediatr Endocrinol Metab 2010;23:153-158.
37. Fonseca V, Brown R, Hochhauser D, Ginsburg J, Havard CW. Acute adrenal crisis precipitated by thyroxine. Br Med J (Clin Res Ed) 1986;292:1185-1186.
38. Phase 1 Trial of 4 Thyroid Hormone Regimens for Transient Hypothyroxinemia in Neonates of <28 Weeks' Gestation 2009;124:258-268. Epub 2009 Jul 5
39. La Gamma EF, Paneth N. Clinical importance of hypothyroxinemia in the preterm infant and a discussion of treatment concerns. Curr Opin Pediatr 2012;24:172-180.
40. Iijima S. Current knowledge of transient hypothyroxinemia of prematurity: to treat or not to treat? J Matern Fetal Neonatal Med 2019;32:2591-2597. Epub 2018 Feb 22
41. Fisher DA. Euthyroid low thyroxine (T4) and triiodothyronine (T3) states in prematures and sick neonates. Pediatr Clin North Am 1990;37:1297-1312.
42. Williams FLR, Ogston SA, van Toor H, Visser TJ, Hume R. Williams FL, Ogston SA, van Toor H, Visser TJ, Hume R. Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. J Clin Endocrinol Metab 2005;90:5954-5963. Epub 2005 Aug 16
43. Carrascosa A, Ruiz-Cuevas P, Clemente M, Salcedo S, Almar J. Thyroid function in 76 sick preterm infants 30-36 weeks: results from a longitudinal study. J Pediatr Endocrinol Metab 2008;21:237-243.
44. Yoon SA, Chang YS, Yang M, Ahn SY, Sung SI, Cho HS, Park WS. Effect of levothyroxine supplementation in extremely low birth weight infants with transient hypothyroxinemia of prematurity. Sci Rep 2022;12:9717.

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Relationship of Glucagon-like Peptide 1 and Peptide YY with Catch-up Growth in Children Born Small for Gestational Age

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What is already known on this topic?

Children born small for gestational age (SGA) are at greater risk for insulin resistance, type 2 diabetes mellitus, and cardiovascular disease in adulthood.

What this study adds?

Glucagon-like peptide 1 may be involved in the development of abnormal glucose metabolism in prepubertal children born SGA who experience catch-up growth.

Abstract

Objective: Children born small for gestational age (SGA) are at a greater risk of developing insulin resistance, type 2 diabetes, and cardiovascular disease in adulthood. Gastrointestinal peptides, some secreted by intestinal L cells, regulate glucose and lipid metabolism and act on the hypothalamus to regulate energy homeostasis. The aim of this study was to explore whether gastrointestinal peptides are involved in metabolic disorders in SGA, which remains unclear.

Methods: The secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) were investigated in prepubertal children born SGA, the differences between catch-up growth and persistent short stature were compared, and correlation with glucose and lipid metabolism was analyzed. GLP-1, PYY, insulin-like growth factor 1, glucose, insulin, and lipid concentrations were analyzed in prepubertal children aged 4-10 years, stratified into three groups: short-SGA (SGA-s), catch-up growth SGA, and normal growth appropriate for gestational age (AGA).

Results: Fasting GLP-1 and PYY concentrations were significantly lower in the SGA group than in the AGA group ($p < 0.05$), and the GLP-1 level in infants born SGA with catch-up growth was lower than that in the SGA-s group ($p < 0.05$). In the SGA population, GLP-1 showed a weak negative correlation with catch-up growth ($r = -0.326$) and positive correlation with fasting insulin ($r = 0.331$).

Conclusion: Lower GLP-1 concentrations may be associated with abnormal glucose metabolism in prepubertal children born SGA with catch-up growth. This is indirect evidence that impaired intestinal L cell function may be involved in the development of metabolic complications in SGA children.

Keywords: Small for gestational age, catch-up growth, glucagon-like peptide 1, peptide YY

Introduction

Small for gestational age (SGA) is defined as a birth length (BL) and/or birth weight (BW) of at least two standard deviations (SDs) below the mean for gestational age according to sex-specific reference values (1). More than 85% of children born SGA have catch-up growth and rapid weight gain by two years of age (2). Catch-up growth is associated with the development of insulin resistance (IR), type 2 diabetes mellitus (T2DM), and cardiovascular disease in adulthood (3). However, the mechanisms underlying the high risk of metabolic outcomes in SGA remain unclear (4).

The human gastrointestinal tract is the first contact for ingested food and is the largest endocrine organ in the human body. Gastrointestinal hormones are key regulators of appetite, energy, and glucose homeostasis (5). Therapeutics for treating T2DM and obesity, based on gut hormones, act

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by enhancing the function of intestinal L cells, indicating the importance of L cells in energy homeostasis. Glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), secreted by intestinal L cells, can delay gastric emptying, suppress appetite, and reduce energy intake (6). The therapeutic combination of GLP-1 and PYY3-36 has demonstrated synergistic effects on energy intake in humans (7). GLP-1 can also promote insulin secretion, inhibit glucagon secretion, enhance pancreatic β-cell proliferation, and have cardioprotective and neuroprotective effects (8).

However, few studies (9,10,11) have focused on gastrointestinal hormone levels in children born SGA. The data are unclear about whether catch-up growth leads to inappropriate secretion of gastrointestinal peptides and whether they are involved in the long-term metabolic outcomes in children born SGA. Abdominal obesity, dyslipidemia, hypertension, and IR have been observed in some children born SGA as early as in the first decade of life (12).

The aim of this study was to explore whether GLP-1 and PYY, two important gastrointestinal hormones secreted by intestinal L cells, are involved in the development of metabolic complications in children born SGA. As some studies have reported that PYY and GLP-1 concentrations decrease after puberty (13), we selected prepubertal children aged 4-10 years as study participants. Furthermore, adults born SGA with normal height have lower insulin sensitivity than short adults born SGA and adults born appropriate for gestational age (AGA) (14). Therefore, we divided the children born SGA into the catch-up and persistently short groups to explore the role of catch-up growth.

Methods

This study was approved by the Ethical Committee of Shenzhen Children's Hospital in Shenzhen, China (no: 202110002, date: 10.18.2021) and was conducted according to the Declaration of Helsinki. Informed consent was obtained from the parents of the participants.

We recruited children born SGA with short stature who were outpatients at the Endocrinology Department at Shenzhen Children's Hospital. Children with normal height were recruited from the Child Healthcare Department at Shenzhen Children's Hospital (Figure 1). The inclusion criteria for the study were: 1) children born at term (37-41 weeks of gestation) and singleton pregnancy; 2) children aged 4-10 years who were in the prepubertal period (defined as Tanner stage 1, without premature thelarche, pubarche, or menarche); and 3) for the SGA group, BW or BL below -2.0 SD for gestation and sex, according to the Chinese standard reference (15) and, for the AGA group, BW and BL between -2.0 SD and +2.0 SD. The exclusion criteria were: maternal gestational diabetes; alcohol abuse, or drug addiction. Furthermore, children with Turner syndrome or Silver-Russell syndrome were excluded.

The SGA group was divided into the short-SGA (SGA-s) group or the catch-up growth SGA (SGA-cu) group, according to whether the current height was above -2.0 SD for age, sex, and population. In all short children born SGA, insulin-like growth factor-1 (IGF-1) was measured to exclude growth hormone (GH) deficiency. GH stimulation tests were also performed; the insulin test with a dose of 0.1 U/kg and the levodopa test with 10 mg/kg (maximum: 0.5 g) were used. GH concentration measurements were performed at 0, 30, 60, 90, and 120 min after the test. Children with IGF-1 levels >-2.0 SD for age and normal peak GH values (≥10 ng/mL) were included in the SGA-s group.

All interviews and physical examinations were performed by a pediatric endocrine physician. The pubertal stage evaluation was performed according to the Tanner system (16), and prepubertal children were defined as children at Tanner stage 1.

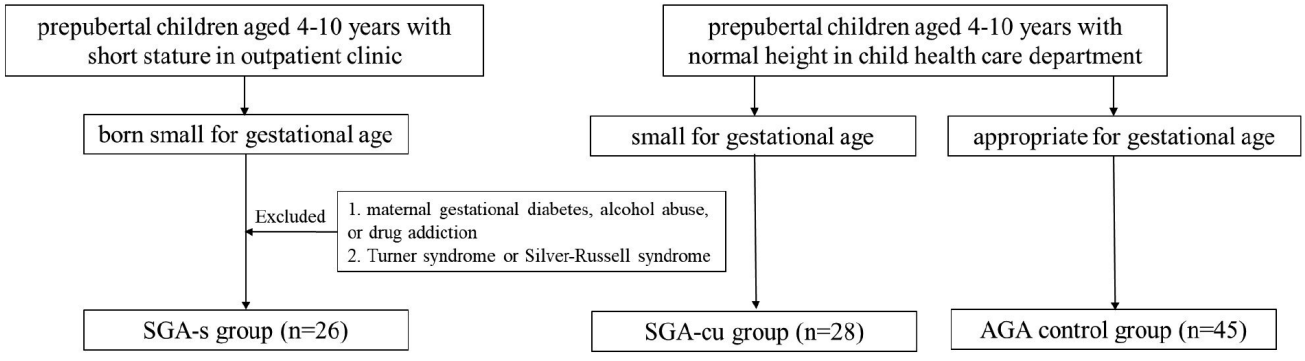

Figure 1. Research design flow chart

SGA: small for gestational age, SGA-cu: catch-up growth SGA, SGA-s: short-SGA, AGA: appropriate for gestational age

The following indices were determined by current height and weight: height SD score (HtSDS), weight SDS (WtSDS), body mass index (BMI), and BMI SDS for chronological age and for height age (HA; the age that corresponds to the child's height when plotted at the 50th percentile on a growth curve). BMI SDS for HA was used to evaluate the nutritional status of the children in each group. The BLSDS and BWSDS were determined using the BL and BW. All parameters were calculated based on Chinese population data (15,17). The parents' heights were measured, the target height (THt) was calculated as mid-parental height minus 6.5 cm for girls and plus 6.5 cm for boys, and the target HtSDS (ThtSDS) was calculated. HtSDS-ThtSDS represents the difference between the current height and target height; ΔHtSDS and ΔWtSDS represent the difference between the current height/weight and BL/weight, respectively.

On the morning of the interview day, fasting serum samples were obtained to measure IGF-1, fasting blood glucose (FBG), fasting insulin (FINS), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), GLP-1, and PYY. Neither a dipeptidyl peptidase-4 inhibitor nor aprotinin was added to the sample. Total serum GLP-1 was measured using a Millipore ELISA kit (Billerica; MA, USA), and total PYY was measured using a Raybiotech ELISA kit (Norcross; GA, USA). The GLP-1 antibody specifically binds to GLP-1 (7-36 and 9-36) with no significant cross-reactivity with GLP-2, GIP, glucagon, or oxyntomodulin. The intra- and inter-assay percent coefficients of variation (%CVs) were <2% and <10%, respectively; the lower detection limit was 1.5 pM. The PYY antibody binds specifically to PYY (1-36 and 3-36). The intra- and inter-assay %CVs were <3% and <6%; the lower detection limit was 1.4 pg/mL. The IGF-1 SDS was calculated based on reference values (18). According to FBG and FINS levels, the quantitative insulin sensitivity check index ($QIUCKI = \frac{1}{log(FINS)+log\ (FBG\,(mmol/L)\times 18)}$) was calculated.

Statistical Analysis

The Shapiro-Wilk test was used to assess variable distribution. Variables fitting a normal distribution are described by mean ± SD, and variables that did not fit a normal distribution were described in quartiles. For normally distributed variables with homogeneous variance, one-way analysis of variance (ANOVA) was performed, with the subsequent use of a *post-hoc* test to assess statistical differences between the groups. Non-normally distributed variables were analyzed using the Kruskal-Wallis test, and differences between the groups were tested using the Mann-Whitney U test. Correlations were evaluated using the Spearman rank correlation coefficient. The Spearman rank correlation coefficient was used to evaluate the correlation of different variables, including auxological data, glucose, lipid, and gastrointestinal hormones, in children born SGA. The HtSDS and THtSDS were compared using paired t-tests for each group. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS)

Table 1. Clinical patient characteristics by group

Group	SGA-s	SGA-cu	AGA	Analysis of variance, p
n (girls/boys)	26 (15/11)	28 (17/11)	45 (13/32)	**0.010**
CA, years	7.70 ± 2.99	8.05 ± 2.67	6.72 ± 2.06	0.082
GA, weeks	38.73 ± 1.31	38.92 ± 1.56	39.11 ± 1.30	0.457
BL, cm	47.63 ± 2.48^{b}	47.78 ± 2.43^{c}	50.33 ± 1.02^{b,c}	**<0.001**
BLSDS	-0.99 ± 1.11^{b}	-0.97 ± 1.12^{c}	0.33 ± 0.58^{b,c}	**<0.001**
BW, kg	2.45 ± 0.30^{b}	2.50 ± 0.29^{c}	3.36 ± 0.37^{b,c}	**<0.001**
BWSDS	-2.03 ± 0.58^{b}	-2.17 ± 1.42^{c}	0.22 ± 0.95^{b,c}	**<0.001**
THtSDS	-1.19 ± 0.74^{a,b}	-0.77 ± 0.76^{a,c}	-0.21 ± 0.67^{b,c}	**0.001**
HtSDS	-2.34 ± 0.35^{a,b}	-0.88 ± 0.77^{a,c}	-0.16 ± 0.97^{b,c}	**<0.001**
HtSDS-THtSDS	-1.15 ± 0.68^{a,b}	-0.14 ± 0.88^{a}	0.04 ± 0.90^{b}	**<0.001**
ΔHtSDS	-1.62 ± 1.13^{a,b}	-0.08 ± 1.33^{a}	-0.48 ± 1.01^{b}	**<0.001**
WtSDS	-1.73 ± 0.73^{a,b}	-0.86 ± 0.69^{b,c}	0.02 ± 0.96^{a,c}	**<0.001**
ΔWtSDS	0.30 ± 1.07^{a}	1.30 ± 1.50^{a,c}	-0.20 ± 1.17^{c}	**<0.001**
BMI-SDS for HA	-0.34 ± 1.17^{b}	-0.34 ± 0.77^{c}	0.24 ± 0.94^{b,c}	**0.015**

*The chi-square test assesses the difference in male and female composition among the three groups. Values in the same row with different superscripts are significantly different: $^{a,b,c}p < 0.05$. Comparisons between groups are classified as follows: a) SGA-s vs. SGA-cu, b) SGA-s vs. AGA, and c) SGA-cu vs. AGA.
SGA: small for gestational age, AGA: appropriate for gestational age, CA: chronological age, GA: gestational age, BL: birth length, BW: birth weight, BLSDS: birth length standard deviation score, BWSDS: birth weight standard deviation score, THtSDS: target height standard deviation score, HtSDS: height standard deviation score, ΔHtSDS: gain in height standard deviation score, ΔWtSDS: gain in weight standard deviation score, BMI-SDS for HA: body mass index standard deviation score for height age, SGA-cu: catch-up growth SGA, SGA-s: short-SGA

software, version 22.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed when $p < 0.05$.

Results

The study included 99 prepubertal children aged 4-10 years, divided into the SGA-s (n = 26), SGA-cu (n = 28), and AGA (n = 45) groups. The characteristics of the groups and anthropometric parameters of the participants in each group are presented in Table 1. The BW and BL of children born SGA, as to be expected, were significantly less than those of the AGA control group. The THt of the SGA-s group was significantly less than that of the SGA-cu group. In the SGA-s group, the HtSDS was significantly less than the THtSDS ($p < 0.0001$); the HtSDS was similar to the THtSDS in the SGA-cu ($p = 0.501$) and AGA ($p = 0.741$) groups. The ΔHtSDS values of the SGA-cu and AGA groups were similar; the ΔWtSDS of the SGA-cu group was significantly greater than that of the AGA control group. The BMI SDS for HA in the SGA-s group was similar to that of the SGA-cu group ($p = 0.938$) and was significantly less than that of the AGA control group ($p = 0.015$).

FBG levels were greater in the SGA-cu group than in the SGA-s and AGA control groups (Table 2). Similarly, LDL levels were greater in the SGA-s group than in the SGA-cu and AGA control groups. However, GLP-1 concentration in the SGA-cu group was significantly lower than in the SGA-s and AGA control groups. SGA individuals, both the catch-up and short groups, had significantly lower concentrations of PYY than AGA controls. However, no significant differences were found between the three groups for FINS, QUICKI, TC, TG, and HDL levels.

Due to the significant difference in the sex distribution among the three groups, we compared the concentration of GLP-1, PYY, and other variables between boys and girls in the AGA control group. The analysis found no significant difference between boys and girls in GLP-1 or PYY levels (Table 3). Concurrent analysis of the correlation between age and GLP-1 and PYY levels in AGA individuals found r-values

Table 2. Laboratory data of the individual groups

Group	Short-s	SGA-cu	AGA	p
FBG, mmol/L	4.41 ± 0.36[a]	4.73 ± 0.59[a,c]	4.38 ± 0.48[c]	0.016
FINS, uIU/mL	5.65 (2.9-8.1)	5.50 (3.45-10.18)	4.24 (3.03-7.46)	0.411
QUICKI	0.39 ± 0.05	0.38 ± 0.04	0.40 ± 0.04	0.314
IGF-1 SDS	0.39 ± 1.15	0.60 ± 1.03	0.99 ± 1.02	0.106
TC, mmol/L	4.27 ± 0.85	3.79 ± 0.69	4.16 ± 0.64	0.062
TG, mmol/L	0.79 ± 0.28	0.77 ± 0.30	0.72 ± 0.19	0.537
HDL, mmol/L	1.50 ± 0.30	1.42 ± 0.22	1.53 ± 0.32	0.448
LDL, mmol/L	2.56 ± 0.72[a]	2.04 ± 0.58[a]	2.30 ± 0.59	0.022
GLP-1, pM	15.08 (5.67-20.47)[a]	5.19 (1.5-10.49)[a,c]	20.24 (9.90-28.49)[c]	0.018
PYY, ng/mL	0.46 (0.27-0.58)[b]	0.45 (0.22-0.52)[c]	0.83 (0.59-1.03)[b,c]	< 0.001

Values in the same row with different superscripts are significantly different: [a,b,c]$p < 0.05$. Comparisons between groups are classified as follows: a. SGA-s vs. SGA-cu, b. SGA-s vs. AGA, and c. SGA-cu vs. AGA.
SGA: small for gestational age, AGA: appropriate for gestational age, FBG: fasting blood glucose, FINS: fasting insulin, QUICKI: quantitative insulin sensitivity check index, GLP-1: glucagon-like, PYY: peptide 1 peptide YY, IGF-1: insulin-like growth factor-1, TG: triglyceride, TC: cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, SDS: standard deviation score

Table 3. GLP-1 and PYY concentrations by sex in the AGA group

Sex	Female (n = 13)	Male (n = 32)	p
CA, years	6.65 ± 2.11	7.16 ± 2.21	0.474
BLSDS	0.23 ± 0.97	0.11 ± 0.58	0.620
BWSDS	0.04 ± 1.01	0.42 ± 1.31	0.340
BMI SDS for HA	0.34 ± 1.15	0.39 ± 0.59	0.841
FBG, mmol/L	4.52 ± 0.38	4.37 ± 0.32	0.241
QUICKI	0.39 ± 0.04	0.40 ± 0.05	0.354
GLP-1, pM	20.09 (12.37-25.78)	20.54 (5.65-30.02)	0.764
PYY, ng/mL	0.76 (0.61-0.99)	0.88 (0.52-1.40)	0.585

AGA: appropriate for gestational age, CA: chronological age, BLSDS: birth length standard deviation score, BWSDS: birth weight standard deviation score; BMI SDS for HA: body mass index standard deviation score for height age, FBG: fasting blood glucose, QIUCKI: quantitative insulin sensitivity check index, GLP-1: glucagon-like, PYY: peptide 1 peptide YY

of -0.221 (p = 0.144) and 0.113 (p = 0.530), respectively. The data are presented in Table 4. In SGA children, fasting GLP-1 levels were negatively correlated with catch-up growth (ΔHt-SDS; r = -0.326) and positively correlated with FINS levels (r = 0.331) but no correlations were found in the AGA control group for the same parameters.

Discussion

Individuals born SGA are at an increased risk of IR and obesity, which are associated with catch-up growth; however, the mechanisms are not fully understood. This study measured GLP-1 and PYY levels in children born SGA who achieved catch-up growth. Our analysis revealed that the fasting GLP-1 and PYY levels of the SGA group were significantly low compared with those of the AGA control group. The GLP-1 level of children born SGA with catch-up growth was lower than that of children born SGA without catch-up growth; the GLP-1 concentration correlated with the FINS level.

GLP-1 and PYY gastrointestinal peptides secreted by intestinal L cells can regulate energy metabolism by delaying gastric emptying, enhancing satiety, and reducing food intake. GLP-1 can also promote insulin secretion, enhance glucose sensitivity of islet β cells, and exert cardioprotective and neuroprotective effects (19). Studies regarding GLP-1 and PYY concentrations in individuals born SGA during the prepubertal period after the catch-up process are scarce. In addition, no studies on PYY secretion levels in individuals born SGA have been reported. One study found no significant difference in GLP-1 secretion levels between adults born SGA and AGA (10). We found that in children aged 4-8 years, GLP-1 and PYY levels were lower in the SGA group than in the AGA control group. Gastrointestinal peptides may represent staged changes and have different physiological significance during the full lifecycle of individuals born SGA. For example, circulating gastrointestinal peptides may be involved in the hypothalamic setpoints of appetite and energy expenditure during the neonatal period (9). In diet-induced obese rats, the GLP-1 analog liraglutide can downregulate the body weight setpoints by regulating microglial polarization (20).

A bidirectional relationship exists between obesity and gastrointestinal hormones (21). Patients with obesity and T2DM have lower PYY levels, and PYY secretion is decreased before blood glucose levels become abnormal in children with obesity (22). However, the question of whether GLP-1 levels are higher or lower in patients with obesity and T2DM patients is inconclusive, possibly due to the cohort design. In addition, obesity pathology may change according to age and sex (23). A cross-sectional study of children aged 6-19 years revealed that the fasting GLP-1 level of AGA children with obesity was greater than that of the healthy control group, and GLP-1 was positively correlated with the BMI SDS (24). Our analysis identified that the SGA group had lower BMI SDS and GLP-1 levels than the AGA group, regardless of whether the SGA group achieved catch-up growth. Further, the SGA-cu group had lower GLP-1 levels than the SGA-s group, possibly indicating that GLP-1 plays different roles in obesity pathogenesis in the SGA population.

Animal model research on gastrointestinal peptide secretion in SGA catch-up growth has been rare. Entero-insular axis disorder has been observed in catch-up fat rats fed a high-fat diet; they rapidly developed IR, impaired incretin effect, reduced intestinal L cells, and decreased expression of proglucagon mRNA (25). In Sprague-Dawley rats fed a diet high in fat and sucrose, elevated GLP-1 levels may play a role in normalizing postprandial glycemia and delaying glucose intolerance by protecting pancreatic β cells from apoptosis (26). The reduction in intestinal L cells in catch-

Table 4. Spearman correlation analysis of variables in individuals with SGA

	ΔWtSDS	BMI SDS for HA	FBG	FINS	QUICKI	TC	TG	HDL	LDL	GLP-1	PYY
ΔHt-SDS	0.353*	-0.266	0.280	-0.163	0.129	-0.236	-0.077	-0.134	-0.205	-0.326*	-0.186
ΔWt-SDS		0.587**	0.211	0.363*	-0.380*	-0.037	0.211	-0.248	-0.034	0.041	0.219
BMI SDS for HA			0.238	0.625**	-0.628**	0.031	0.290	-0.117	-0.036	0.102	0.234
FBG				0.538**	-0.601**	0.305	0.288	0.098	0.175	0.013	0.010
FINS					-0.995**	0.226	0.430**	0.084	0.086	0.331*	-0.043
QUICKI						-0.220	-0.422**	-0.078	-0.084	-0.307	0.014
TC							0.477**	0.288	0.860**	0.301	-0.111
TG								-0.237	0.358*	0.025	-0.005
HDL									0.004	0.193	-0.173
LDL										0.287	0.083
GLP-1											-0.021

*p < 0.05; **p < 0.0.
SGA: small for gestational age, FINS: fasting insulin, BMI SDS for HA: body mass index standard deviation score for height age, FBG: fasting blood glucose, QUICKI: quantitative insulin sensitivity check index, GLP-1: glucagon-like, PYY: peptide 1 peptide YY, ΔHtSDS: gain in height standard deviation score, ΔWtSDS: gain in weight standard deviation score, TG: triglyceride, TC: cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, SDS: standard deviation score

up fat rats may indicate inadequate compensatory capacity. A correlation was identified between GLP-1 and catch-up growth (ΔHt-SDS) and FINS in the SGA group, possibly indicating a decreased incretin effect of GLP-1 in children born SGA, an important factor in the development of obesity and T2DM development.

In a lamb model of intrauterine growth restriction, injection of the GLP-1 analog exedin-4 normalized insulin secretion patterns (27), proving the positive effect of GLP-1. However, long-term monitoring and further mechanistic studies in SGA individuals are required to verify these findings. In addition, the THt of the SGA group was less than that of the AGA control group, and the SGA-s group had a lower THt than the catch-up SGA group, consistent with the height of individuals with SGA-s being -1 SD less than that of the AGA control (1). Our study found no significant difference in IGF-1 level (IGF-1 SDS) among the SGA-cu, SGA-s, and AGA control groups. This finding contrasts with previous reports that IGF-1 concentrations were significantly higher in the normal-height SGA group (28), possibly related to the greater BMI in the AGA control group.

Study Limitations

A limitation of our study is that we analyzed only GLP-1 and PYY secretions, and the subsequent physiological effects are unclear. For example, how the incretin effect of GLP-1 changes in SGA individuals and how these changes affect long-term metabolic outcomes are unknown. These issues need to be explored in future studies.

Conclusion

This study tested the levels of two important gastrointestinal peptides, GLP-1 and PYY, in prepubertal children born SGA, and found that the concentrations of fasting GLP-1 and PYY in children born SGA were lower than those of children born AGA. In addition, GLP-1 levels in the SGA-cu group were lower than those in the SGA-s group. GLP-1 levels in children born SGA correlated with catch-up growth and FINS levels. This suggests that impaired intestinal L cell function may be involved in the development of metabolic complications in SGA children.

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Ethics

Ethics Committee Approval: This study was approved by the Ethical Committee of Shenzhen Children's Hospital in Shenzhen, China (no: 202110002, date: 10.18.2021) and was conducted according to the Declaration of Helsinki.

Informed Consent: Informed consent was obtained from the parents of the participants.

Authorship Contributions

Concept: Chun-Xiu Gong, Design: Zhe Su, Data Collection or Processing: Yu-Chuan Li, Analysis or Interpretation: Li Wang, Literature Search: Bing-Yan Cao, Chang Su, Writing: Li Wang.

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References

1. Finken MJJ, van der Steen M, Smeets CCJ, Walenkamp MJE, de Bruin C, Hokken-Koelega ACS, Wit JM. Children Born Small for Gestational Age: Differential Diagnosis, Molecular Genetic Evaluation, and Implications. Endocr Rev 2018;39:851-894.
2. Netchine I, van der Steen M, López-Bermejo A, Koledova E, Maghnie M. New Horizons in Short Children Born Small for Gestational Age. Front Pediatr 2021;9:655931.
3. Goedegebuure WJ, Van der Steen M, Smeets CCJ, Kerkhof GF, Hokken-Koelega ACS. SGA-born adults with postnatal catch-up have a persistently unfavourable metabolic health profile and increased adiposity at age 32 years. Eur J Endocrinol 2022;187:15-26.
4. An J, Wang J, Guo L, Xiao Y, Lu W, Li L, Chen L, Wang X, Dong Z. The Impact of Gut Microbiome on Metabolic Disorders During Catch-Up Growth in Small-for-Gestational-Age. Front Endocrinol (Lausanne) 2021;12:630526.
5. Rehfeld JF. Gastrointestinal hormone research - with a Scandinavian annotation. Scand J Gastroenterol 2015;50:668-679. Epub 2015 Mar 18
6. Koliaki C, Liatis S, Dalamaga M, Kokkinos A. The Implication of Gut Hormones in the Regulation of Energy Homeostasis and Their Role in the Pathophysiology of Obesity. Curr Obes Rep 2020;9:255-271.
7. Boland BB, Laker RC, O'Brien S, Sitaula S, Sermadiras I, Nielsen JC, Barkholt P, Roostalu U, Hecksher-Sørensen J, Sejthen SR, Thorbek DD, Suckow A, Burmeister N, Oldham S, Will S, Howard VG, Gill BM, Newton P, Naylor J, Hornigold DC, Austin J, Lantier L, McGuinness OP, Trevaskis JL, Grimsby JS, Rhodes CJ. Peptide-YY3-36/glucagon-like peptide-1 combination treatment of obese diabetic mice improves insulin sensitivity associated with recovered pancreatic β-cell function and synergistic activation of discrete hypothalamic and brainstem neuronal circuitries. Mol Metab 2022;55:101392. Epub 2021 Nov 12
8. Müller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, Fritsche A, Gribble F, Grill HJ, Habener JF, Holst JJ, Langhans W, Meier JJ, Nauck MA, Perez-Tilve D, Pocai A, Reimann F, Sandoval DA, Schwartz TW, Seeley RJ, Stemmer K, Tang-Christensen M, Woods SC, DiMarchi RD, Tschöp MH. Glucagon-like peptide 1 (GLP-1). Mol Metab 2019;30:72-130. Epub 2019 Sep 30
9. Díaz M, Bassols J, Sebastiani G, López-Bermejo A, Ibáñez L, de Zegher F. Circulating GLP-1 in infants born small-for-gestational-age: breast-feeding versus formula-feeding. Int J Obes (Lond) 2015;39:1501-1503. Epub 2015 Jun 19
10. Brøns C, Saltbæk PN, Friedrichsen M, Chen Y, Vaag A. Endocrine and metabolic diurnal rhythms in young adult men born small vs appropriate for gestational age. Eur J Endocrinol 2016;175:29-40.

11. Díaz M, García-Beltran C, López-Bermejo A, de Zegher F, Ibáñez L. GLP-1 and IGF-I levels are elevated in late infancy in low birth weight infants, independently of GLP-1 receptor polymorphisms and neonatal nutrition. Int J Obes (Lond) 2018;42:915-918. Epub 2017 Nov 1
12. Blusková Z, Koštálová L, Celec P, Vitáriušová E, Pribilincová Z, Maršálková M, Šemberová J, Kyselová T, Hlavatá A, Kovács L. Evaluation of lipid and glucose metabolism and cortisol and thyroid hormone levels in obese appropriate for gestational age (AGA) born and non-obese small for gestational age (SGA) born prepubertal Slovak children. J Pediatr Endocrinol Metab 2014;27:693-699.
13. Horner K, Lee S. Appetite-related peptides in childhood and adolescence: role of ghrelin, PYY, and GLP-1. Appl Physiol Nutr Metab 2015;40:1089-1099. Epub 2015 Aug 6
14. Cho WK, Suh BK. Catch-up growth and catch-up fat in children born small for gestational age. Korean J Pediatr 2016;59:1-7. Epub 2016 Jan 2
15. Capital Institute of Pediatrics; Coordinating Study Group of Nine Cities on the Physical Growth and Development of Children. [Growth standard curves of birth weight, length and head circumference of Chinese newborns of different gestation]. Zhonghua Er Ke Za Zhi 2020;58:738-746.
16. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170-179.
17. Li H, Ji CY, Zong XN, Zhang YQ. [Height and weight standardized growth charts for Chinese children and adolescents aged 0 to 18 years]. Zhonghua Er Ke Za Zhi 2009;47:487-492.
18. Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, Körner A, Obermayer-Pietsch B, Hübener C, Dahlgren J, Frystyk J, Pfeiffer AF, Doering A, Bielohuby M, Wallaschofski H, Arafat AM. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. J Clin Endocrinol Metab 2014;99:1712-1721. Epub 2014 Feb 27
19. Hira T, Pinyo J, Hara H. What is GLP-1 really doing in obesity? Trends Endocrinol Metab 2020;31:71-80. Epub 2019 Oct 18
20. Liao T, Zhang SL, Yuan X, Mo WQ, Wei F, Zhao SN, Yang W, Liu H, Rong X. Liraglutide Lowers Body Weight Set Point in DIO Rats and its Relationship with Hypothalamic Microglia Activation. Obesity (Silver Spring) 2020;28:122-131. Epub 2019 Nov 26
21. Ribeiro FM, Silva MA, Lyssa V, Marques G, Lima HK, Franco OL, Petriz B. The molecular signaling of exercise and obesity in the microbiota-gut-brain axis. Front Endocrinol (Lausanne) 2022;13:927170.
22. Roth CL, Bongiovanni KD, Gohlke B, Woelfle J. Changes in dynamic insulin and gastrointestinal hormone secretion in obese children. J Pediatr Endocrinol Metab 2010;23:1299-1309.
23. Farhadipour M, Depoortere I. The Function of Gastrointestinal Hormones in Obesity-Implications for the Regulation of Energy Intake. Nutrients 2021;13:1839.
24. Stinson SE, Jonsson AE, Lund MAV, Frithioff-Bøjsøe C, Aas Holm L, Pedersen O, Ängquist L, Sørensen TIA, Holst JJ, Christiansen M, Holm JC, Hartmann B, Hansen T. Fasting Plasma GLP-1 Is Associated With Overweight/Obesity and Cardiometabolic Risk Factors in Children and Adolescents. J Clin Endocrinol Metab 2021;106:1718-1727.
25. Zheng J, Xiao KL, Chen L, Wu C, Hu X, Zeng T, Chen XQ, Li WJ, Deng X, Li H, Li YM. Insulin sensitizers improve the GLP-1 secretion and the amount of intestinal L cells on high-fat-diet-induced catch-up growth. Nutrition 2017;39-40:82-91. Epub 2017 Jan 12
26. Pinyo J, Hira T, Hara H. Continuous feeding of a combined high-fat and high-sucrose diet, rather than an individual high-fat or high-sucrose diet, rapidly enhances the glucagon-like peptide-1 secretory response to meal ingestion in diet-induced obese rats. Nutrition 2019;62:122-130. Epub 2019 Jan 11
27. Gatford KL, Sulaiman SA, Mohammad SN, De Blasio MJ, Harland ML, Simmons RA, Owens JA. Neonatal exendin-4 reduces growth, fat deposition and glucose tolerance during treatment in the intrauterine growth-restricted lamb. PLoS One 2013;8:e56553. Epub 2013 Feb 12
28. Stawerska R, Szałapska M, Hilczer M, Lewiński A. Ghrelin, insulin-like growth factor I and adipocytokines concentrations in born small for gestational age prepubertal children after the catch-up growth. J Pediatr Endocrinol Metab 2016;29:939-945.

ORIGINAL ARTICLE

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Clinical Variability in a Family with Noonan Syndrome with a Homozygous *PTPN11* Gene Variant in Two Individuals

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What is already known on this topic?

Noonan syndrome (NS) is an inherited multisystem disease, with a reported prevalence of 1/1000-2500. The most common findings in NS are short stature, distinctive facial features (ptosis, hypertelorism, low-set ears, low hairline), mane neck, chest deformity, bleeding diathesis, cryptorchidism, learning disability, congenital heart defects and varying levels of intellectual disability. While NS is inherited in an autosomal dominant manner, it is noteworthy that approximately half of the cases manifest sporadically.

What this study adds?

A previously described variant in *PTPN11* was present in nine members of two consanguineous families of the same kindred and was inherited in a homozygous fashion in two members. To the best of our knowledge, these are the first published homozygous *PTPN11* cases. The patients with homozygous inheritance had lower height standard deviation scores than family members with heterozygous inheritance.

Abstract

Objective: Noonan syndrome (NS) is characterized by dysmorphic facial features, short stature, congenital heart defects, and varying levels of developmental delays. It is a genetic, multisystem disorder with autosomal dominant inheritance and is the most common of the RASopathies. In approximately 50% of patients, NS is caused by variants in the Protein Tyrosine Phosphatase Non-Receptor Type 11 (*PTPN11*) gene. The aim of this study was to evaluate two patients with a previously reported *PTPN11* homozygous variant for the first time and seven other kindred members carrying the same heterozygous variant in terms of clinical, biochemical, genetic, and response to treatment.

Methods: Nine patients diagnosed with NS due to the same variants in the *PTPN11* gene were included in the study.

Results: The median (range) age at diagnosis was 11.5 (6.8-13.9) years and the mean follow-up duration was 4.7 (1-7.6) years. In eight patients (88.9%), short stature was present. The height standard deviation score of the patients on admission was -3.24 ± 1.15. In six of the patients, growth hormone treatment was initiated. Cardiovascular or bleeding disorders were not detected in any of the patients. Three (33.3%) had hearing loss, two (22.2%) had ocular findings and one (11.1%) had a horseshoe kidney. The mean psychomotor development performance score was 84.03 ± 17.09 and the verbal score was 82.88 ± 9.42. Genetic analysis revealed a variant in the *PTPN11* gene [c.772G > A; (p.Glu258Lys)] that had been previously described and was detected in all patients. Two patients were homozygous for this variant and short stature was more severe in these two.

Conclusion: A previously described in *PTPN11* affected nine members of the same kindred, two with homozygous inheritance and the remainder being heterozygous. To the best of our knowledge, these are the first homozygous *PTPN11* case reports published, coming from two related consanguineous families.

Keywords: *PTPN11*, Noonan syndrome, short stature

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Introduction

Noonan syndrome (NS) is an inherited disease that affects many different systems, and its prevalence has been reported as 1/1000-2500 (1,2). The most common findings in NS are short stature, distinctive facial features (ptosis, hypertelorism, low-set ears, low hairline), mane neck, chest deformity, bleeding diathesis, cryptorchidism, learning disability, congenital heart defects, and varying levels of intellectual disability (2).

Although NS is inherited in an autosomal dominant pattern in a significant proportion of patients, it occurs sporadically in approximately half of the patients. NS is caused by variants in genes that encode proteins in the RAS-Mitogen-Activated Protein Kinase (RAS-MAPK) pathway. The RAS-MAPK pathway is vital for many cellular functions such as proliferation and differentiation (3). Many genes including *PTPN11, SOS1, KRAS, RAF1, NRAS, BRAF, SHOC2,* and *RIT1* have been identified that may cause NS. The use of next-generation sequencing (NGS) techniques has improved the identification of new genes and increased the rate of reaching a molecular genetic diagnosis in patients (4,5). The Protein Tyrosine Phosphatase Non-Receptor Type 11 (*PTPN11*) gene (MIM*176876) is located in the chromosome 12q24.13 region and comprises 16 exons. The first described phenotype caused by pathogenic/likely pathogenic alterations in this gene, which has been implicated in the genetic etiology in 50-60% of individuals with NS, is called NS type 1. In individuals with *PTPN11* variants, short stature is observed more often due to resistance to growth hormone (GH) (6,7). However, the pathophysiology of short stature in patients with NS is not yet fully understood. Various likely mechanisms have been postulated. In terms of GH, it has been suggested that GH deficiency, GH neurosecretory dysfunction, or mild GH resistance may be present. *PTPN11* encodes SHP2, a cytoplasmic protein tyrosine phosphatase that positively regulates RAS signaling. SHP2 binds to its phosphotyrosyl-containing signaling partners through the SH2 domain, which controls its sub-cellular localization and functional regulation. While alterations in the *PTPN11* gene are observed more frequently in patients with pulmonary stenosis and short stature, they are very rare in patients with hypertrophic cardiomyopathy (HCM) and/or severe intellectual disability. It has been shown that the type of variants are mostly missense and these affect the function of SHP2 through different mechanisms (8). Children with NS are prone to juvenile myelomonocytic leukemia (JMML), and somatic variants in the *PTPN11* gene have been detected in approximately one-third of children with non-syndromic JMML and in patients with other childhood myeloid and lymphoid malignancies at varying rates.

Methods

Nine patients who presented to Diyarbakır Children's Hospital Pediatric Endocrinology Outpatient Clinic and were diagnosed with NS due to a variant in the *PTPN11* gene were included in the study. There was consanguinity between the parents in all patients. The presenting complaints, age, body weight (BW), height, BW and height standard deviation scores (SDS), maternal and paternal height, bone age, and detailed physical examination findings of the participants included in the study were recorded. In all patients, ophthalmology examinations and hearing tests were performed. All patients underwent echocardiography performed by an experienced pediatric cardiologist to detect cardiovascular pathologies. Kent EGY and Porteus Maze tests were used to evaluate the neurocognitive functions of all patients. Abdominal ultrasound was requested to detect other accompanying pathologies. Complete blood count, prothrombin time, and activated partial thromboplastin time tests were requested for hematologic disorders that may accompany NS. *PTPN11* gene analysis was requested in all patients. In addition, informed consent was obtained from all patients included in the study.

Molecular Analysis

After written consent was obtained from the families of the affected individuals, molecular analyses were planned. To determine the genetic etiology, molecular analyses were performed by using a targeted NGS panel (TruSight One Sequencing Panel by Illumina). Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA) was used according to the manufacturer's instructions for target enrichment. Paired-end sequencing was performed on all samples using the Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA). NGS data were analyzed using the Illumina VariantStudio software and Integrative Genomics Viewer (IGV) (https://www.igv.org/). The frequency of the identified variant was investigated in different databases: NCBI dbSNP build141 (http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), and Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/). The impact of the variant on protein structure was evaluated using several *in silico* prediction tools such as SIFT, MutationTaster, and PolyPhen. American College of Medical Genetics (ACMG) guidelines were used for the classification of pathogenicity (9). Segregation analysis was performed using the Sanger Sequencing method.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences, version 21 (IBM Inc., Armonk, NY, USA). Data of each affected patient are presented in Table 1. Descriptive statistics are given in the Results section. Non-parametic data as median (interquartile range 25th-75th percentile).

Results

A total of nine patients from four consanguineous families were included in the study. Four of the patients were male and five were female (Figure 1). There was consanguinity between the parents in all four families. The median (range) age at diagnosis was 11.5 (6.8-13.9) years, and the median follow-up duration was 4.7 (1.0-7.6) years. The most common clinical finding was short stature, present in eight of the patients (88.9%). Dysmorphic manifestations of NS, including broad forehead, ptosis, down-slanting palpebral fissures, and low-set ears, were present in seven (77.8%) of nine patients. Cardiovascular pathologies were not detected in any of the patients. Three (33.3%) of the patients had hearing loss, two (22.2%) had astigmatism and one (11.1%) had a horseshoe kidney. One of the four male patients (25%) had cryptorchidism. The mean psychomotor development performance score of the patients was 84.03 ± 17.09 and the mean verbal score was 82.88 ± 9.42 (90-110). No bleeding disorder was detected in any of the patients. The patients exhibited the stigmata of NS clinically. Variants in the *PTPN11* gene were detected in all patients. Homozygous variants were detected in two patients (one female and one male), and heterozygous variants were detected in the other seven patients.

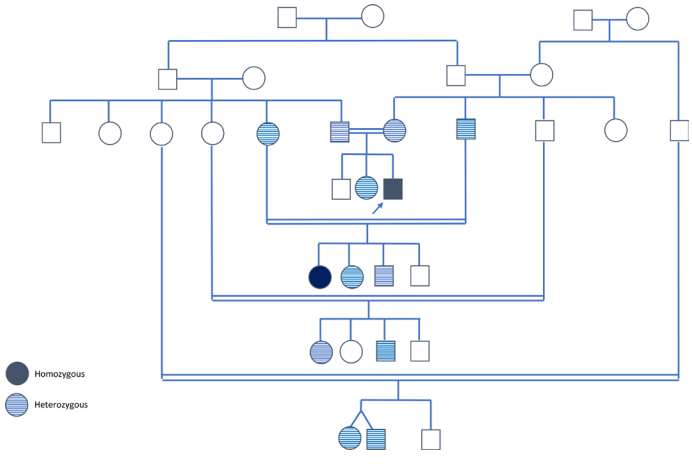

Figure 1. Pedigree of the proband (arrow). Genetic analyses could be performed in the 9 affected children and in the parents of heterozygous cases

The first homozygous case (1a) presented with short stature at the age of 9.4 years. He was born at 39 weeks with a birth weight of 2000 g. The patient did not have any prenatal and pre-school complaints but had school failure. At initial presentation his height was 110 cm (-4.28 SDS) and his weight was 17 kg (-4.16 SDS). Dysmorphology assessment identified bilateral proptosis especially prominent in the right eye, hypertelorism, prominent and wide nasal bridge, low-hanging columella, high-arched palate, dimple on the chin, and low-set ears accompanied by bilateral helical rim deformity. In addition, flaring of the outer one third of both eyebrows, low posterior hairline and mane neck were detected. He had no cardiac, hearing and vision problems, and he had borderline intellectual functioning. He had been operated on for bilateral cryptorchidism at the age of 1.5 years. GH therapy was initiated for severe short stature and poor growth rate. The treatment was terminated at the end of the third year due to a poor response to GH and non-compliance with the treatment. On follow-up, it was found that puberty had not yet started in this male patient with homozygous variant, even though he was 16 years old. We believe this is the first case of NS with homozygous *PTPN11* variant published.

The second case (IVa) with a homozygous variant also presented due to short stature, at the age of 12.6 years. Patient Ia and this patient were cousins and she also similarly had a history of consanguinity between her parents. She was born at 38 weeks with a birth weight of 2000 g. She had poor school performance. At initial presentation her height was 124 cm (-5.3 SDS) and her weight was 23 kg (-4.33 SDS). Dysmorphology assessment again identified prominent and wide nasal bridge, low-hanging columella and low-set ears, helical rim deformity, hypertelorism, high-arched palate, low posterior hairline, mane neck, and chest deformity. Similar to her cousin, she had mild ptosis in the right eye and a dimple on the chin. She also did not have cardiac and hearing problems, and she had borderline intellectual functioning. Eye examination revealed the presence of astigmatism. Puberty started spontaneously at the age of 13.8 years. On follow-up, GH therapy was initiated for severe short stature and poor growth rate at the age of 13.5 years. The treatment was terminated at the age of 16.1 years when the patient reached the height of 145 cm (-3.07 SDS) due to non-compliance with the treatment and epiphyseal closure.

In cases 1a and 4a, molecular analysis revealed homozygous c.772G>A variants in *PTPN11* (NM_001330437.2) (Figure 2). This missense variant resulted in the substitution of glutamine for lysine at the 258th position of *PTPN11* mRNA. The CADD score at this position was 41. This alteration

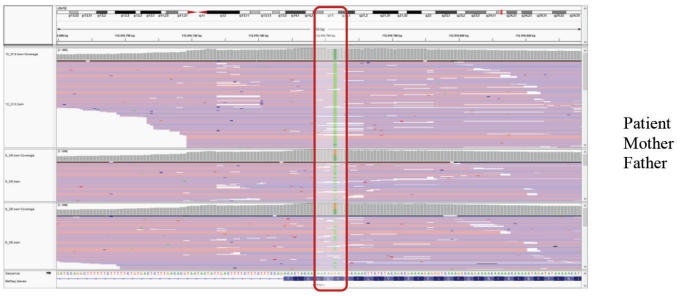

Figure 2. Homozygous *PTPN11* variant [NM_001330437.2; c.772G > A, (p.Glu258Lys)] in proband and heterozygous carrier status in parents visualized on IGV

IGV: integrative genomics viewer

was not found in the GnomAD or in public databases. However, it was submitted to ClinVar as likely pathogenic, and according to ACMG 2015 guidelines, it was pathogenic. However, the impact of this amino acid substitution on the structure and function of the protein was predicted to be benign, according to the Polyphen2 prediction tool. Both parents and other affected family members were found to be heterozygous for the same variant.

Height SDS scores of the two patients with homozygous variants were found to be lower than the other patients (Table 1). Puberty started at the age of 13.8 years in the female patient with a homozygous variant, but did not start in the male patient, even though he was 16 years old. The clinical and laboratory data of all patients were summarized in Table 1.

Discussion

NS is characterized by short stature, hypertelorism, ptosis, mane neck, low-set ears, high-arched palate, chest deformities, cryptorchidism, bleeding disorders, learning disability, and cardiovascular anomalies. Multiple genes are involved in the etiology of NS. Thus, there are 14 different types of NS based on the hereditary traits and genes responsible for the etiology (10). In the present study, nine patients, all diagnosed with NS due to the [c.772G > A(p.Glu258Lys)] variant in the *PTPN11* gene, which has been described previously, are presented (11). Bowling et al. (11) reported that a male patient diagnosed with NS carried variants in *LTZR1* (NM_006767.4: c.742G > A; p.Gly248Arg) and *PTPN11* (NM_002834.5:c.772G > A; p.Glu258Lys) genes, which were shown to be inherited from his parents. However, the *LTZR1* variant was accepted as a causative variant for the disease that had been previously reported as pathogenic. SHP2 is a cytoplasmic protein-tyrosine phosphatase that is essential for vertebrate development. Most of the *PTPN11* biallelic variants show a lethal effect. In animal studies, it was shown that SHP2 has an essential role in early development in the mouse, and homozygous *PTPN11-/-* embryos died pre-implantation (12). We postulate that the biallelic *PTPN11* variant detected in the two affected individuals in this study have a lesser effect on the protein function, as predicted by Polyphen2 (benign protein effect). These cases are the first published human cases of NS with homozygous *PTPN11* variants. We suggest that it is possible to encounter homozygous rare variants in families due to the high rate of consanguineous marriages in Turkey. Of note, short stature was more severe in the two patients with homozygous variants.

Proportionate short stature is the most common presenting complaint in patients with NS (13). Despite normal birth weight and height, short stature is observed in 50-70% of patients (7). The median age of onset of puberty in patients with NS has been reported as 13.4 (10.8-16.4) years in boys and 13 (10.9-15) years in girls (14). Short stature was present in eight of the nine patients (88.9%) in this study, which was slightly higher than the rates reported in the literature. In previous studies, it has been reported that among patients with NS, short stature is more common in children with *PTPN11* gene variants (6,7). Thus, the higher rate of short stature in the present study was possibly due to all patients harboring variants in the *PTPN11* gene.

GH treatment in NS is thought to be beneficial because the dysmorphic findings are similar to those in Turner syndrome. Therefore, in NS patients with severe short stature, GH has been administered at the same dose used in the treatment of Turner syndrome (15,16). In the multicenter study of MacFarlane et al. (17), recombinant human GH (rhGH) treatment (0.33 mg/kg/week) was administered to 23 NS patients with a mean age of 9.3 years. The mean height SDS of the patients was -2.7 ± 0.4 at the start of treatment, which reached -1.9 ± 0.9 at the end of three years. In the study of Şıklar et al. (18), which was the first multicenter study on NS reported in Turkey, 124 patients with NS were evaluated retrospectively and 47 (37.9%) with a clinical diagnosis (according to the Van Der Burgt Criteria) or genetic diagnosis received rhGH treatment. While the height SDS of the patients was -3.62 ± 1.14 at the start of GH treatment, it reached -2.85 ± 0.96 after three years of treatment. In the present study, GH treatment was initiated at a dose of 0.023 mg/kg/week in six of the nine patients. In patients who received GH treatment, while the initial mean height SDS was -3.68 ± 1.13, it had improved to -3.28 ± 0.94 after one year of treatment. Both the mean height SDS on admission of all patients and the post-treatment height SDS

Table 1. Clinical and laboratory findings of patients with c.772G > A(p.Glu258Lys) mutation in the *PTPN11* gene

	I		II		III		IV		
	a	b	a	b	a	b	a	b	c
Genetic mutation	Homozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Homozygous	Heterozygous	Heterozygous
Birth weight (gr)	2000	2800	3100	3000	1600	1700	2000	2900	3100
Gestational age (week)	39	39	39	38	37	37	38	39	39
Gender	Male	Female	Male	Female	Male	Female	Female	Female	Male
Age at diagnosis (years)	9.4	12	6.8	11	9.3	13.1	12.6	13.9	12.4
Height on admission (SDS) Weight on admission (SDS)	110 (-4.28) 17 (-4.16)	131 (-3.44) 27 (-2.8)	102.4 (-3.39) 14.7 (-2.24)	135.4 (-1.68) 29.4 (-1.33)	121 (-2.06) 23.4 (-1.51)	143.2 (-2.49) 41.5 (-1.31)	124 (-5.3) 23 (-4.33)	136.4 (-3.91) 31.7 (-3.7)	133 (-2.36) 28 (-2.3)
Maternal height (cm) Paternal height (cm) Target height (cm)	140 165 159	140 165 146	158 166 168.5	158 166 155.5	146.5 167 163.3	146.5 167 150.3	152 164 151.5	152 164 151.5	152 164 164.5
Typical facial findings									
- Mane neck	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
- Ptosis	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes
- Chest deformity	No	No	Yes	No	No	No	Yes	No	No
Cardiovascular anomalies	No	No	No	No	No	No	No	No	No
GUS anomalies									
- Cryptorchidism	Yes	(-)	No	(-)	No	(-)	(-)	(-)	No
- Renal abnormalities	No	No	No	No	Horseshoe Kidney	No	No	No	No
Abnormal hemostasis	No	No	No	No	No	No	No	No	No
Ocular anomalies	No	No	Yes (astigmatism)	No	No	No	Yes (astigmatism)	No	No
Cutaneous findings	No	No	No	No	No	No	No	No	No
Neurodevelopmental disorders									
- Performance	61	87	81	79	82	76	71	105	117
- Verbal	71	89	90	87	90	71	70	85	93
Hearing loss	No	No	No	Conductive	Conductive	Conductive	No	No	No
IGF-1 (SDS) IGFBP-3 (SDS)	45 (-2.33) 2190 (-1.51)	148 (-1.17) 4200 (-0.69)	103 (0.33) 3700 (0.49)	250 (0.03) 3450 (-1.01)	120 (-0.38) 4200 (0.33)	413 (0.32) 4262 (-0.76)	151 (-1.15) 2110 (-1.98)	241 (-0.88) 4407 (-0.67)	109.5 (-1.49) 2684 (-1.66)
Bone age	6	11.5	5	11	7.5	12.5	10.5	11.5	10.6
Height at growth	Received GH therapy	Did not receive GH therapy	Received GH therapy	Did not receive GH therapy	Received GH therapy	Did not receive GH therapy	Received GH therapy	Received GH therapy	Received GH therapy
Hormone therapy start (SDS) Height after one year of growth hormone therapy (SDS)	115 (-4.31) 122 (-3.96)		113 (-2.9) 119 (-2.57)		137.6 (-2.7) 146.3 (-2.4)		126.2 (-5.52) 133 (-4.77)	137 (-3.97) 142 (-3.39)	133.4 (-2.7) 141 (-2.59)

SDS: standard deviation score, GUS: genitourinary system, IGF-1: insulin-like growth factor-1, IGFBP-3: insulin-like growth factor binding protein-3, GH: growth hormone

of the patients who received GH treatment in the present study were consistent with the literature. In contrast to the literature, the height SDS of the patients with homozygous variants (Ia: -4.28 and IVa: -5.3 SDS) was much lower than the other seven patients with heterozygous variants (mean height SDS: -2.79 ± 0.80).

The most common cardiac abnormalities seen in patients with NS are pulmonary valve stenosis (PVS) and HCM (19,20). In addition to its association with PVS, *PTPN11* variant was reported to be associated with enlargement of the aortic annulus and aortic root (21). In the study of Bell et al. (22), in which 686 pediatric patients diagnosed with PVS between 2009 and 2019 were evaluated retrospectively, NS was detected in 9%. In the present study, cardiac pathology was not detected in any of the patients.

The ophthalmologic findings of NS include a broad spectrum of mainfestations, such as external ocular malformations, refractive errors, mobility abnormalities, and ocular anterior and posterior segment disorders. One of the main criteria for the diagnosis of NS is facial dysmorphology with external ocular features, consisting of hypertelorism, epicanthic folds, ptosis, and/or down-slanting palpebral fissures (23). Marin et al. (24) showed that the most common finding (74%) in patients with identified *PTPN11* variants was down-slanting palpebral fissures. Refractive errors (astigmatism-myopia-hyperopia) have also been reported to be common ocular findings (23). In patients with refractive errors, *PTPN11* variant has usually been detected (10). In the presented kindred, 22.2% had refractive errors and 77.8% had ptosis, which is consistent with previous studies.

In patients with NS, external ear malformations, conductive hearing loss and, less frequently, sensorineural hearing loss may be observed (25). A study by van Trier et al. (25) evaluated 34 patients with NS and hearing loss. They found conductive hearing loss caused by otitis media with effusion that occurred in the past in 20 (58.8%) patients, sensorineural hearing loss in nine (26.5%), permanent conductive hearing loss in two (5.9%), and mixed hearing loss in two (5.9%). Conductive hearing loss was present in three (33.3%) of our patients.

Musculoskeletal manifestations of NS consist of insufficient and/or delayed growth causing short stature, and axial skeletal deformities including spine and rib cage abnormalities such as kyphosis, scoliosis, vertebral anomalies, and pectus deformities, together with micrognathia, cubitus valgus, brachydactyly, syndactyly and osteopenia (26). Chest deformities were present in two (22.2%) of our patients.

Neurological, cognitive and behavioral problems of individuals with NS are highly variable. Patients with NS usually have normal intelligence, but 10% to 40% require special education. The IQ score of the patients is reported to vary between 70 and 120 (27). Affected individuals have been shown to have 10 points less IQ than unaffected family members or 1 SD below the general population (28). The heterogeneity observed in cognitive abilities in RAS-MAPK signaling pathway syndromes, including NS, may vary depending on the genes affected and the type of variant. For example, patients with *PTPN11* and *SOS1* variants show mild cognitive delay (27). Speech disorders are more common compared to the general population. In these patients, hearing and neurodevelopmental evaluations should be performed. Similarly, IQ scores of our patients were between 61-117. While the performance score of patients with heterozygous variants was 89.6 ± 15.4 and the verbal score was 86.4 ± 7.25, the performance scores of the two homozygous cases were 61 and 71, respectively, their verbal scores were calculated as 71 and 70. Studies have shown that the *PTPN11* gene plays a role in brain development (29). Similarly, in our study, the majority of our cases had learning disabilities.

Renal abnormalities in NS are usually mild with a reported frequency of 11% (30). The prevalence of cryptorchidism in boys with NS is 60-80% (31). In the present study, cryptorchidism was present in one (25%) of the four male patients and horseshoe kidney pathology was present in one of the nine patients (11.1%). Given the reported frequencies of these two anomalies, all children diagnosed with NS should be evaluated in terms of cryptorchidism and renal pathology.

In different studies on the prevalence of bleeding disorders in NS, the rate has varied from 30-72% (32). In addition, the prevalence of hemostatic laboratory derangements has been reported to vary between 30-74% (32). The prevalence of bleeding disorders has been reported to be 14-60% in patients with *PTPN11* variant (31). Despite all patients in the present study having *PTPN11* variants, and contrary to the literature, no bleeding disorder was found in any of them. Functional studies could not be performed in the patients.

Study Limitations

The follow-up period was short in patients who were started on GH treatment. Due to time constraints and limited resources, functional studies on the effect on the protein could not be conducted, but we hope to investigate this issue in future studies.

Conclusion

NS is a variable syndrome that may affect many systems. All patients diagnosed with NS should be followed up with a multidisciplinary approach. In the present study, a variant that had been described before in the literature was detected in the *PTPN11* gene and, notably, this variant was homozygous in two patients. To the best of our understanding, these are the first human homozygous *PTPN11* case reports published and they come from two related, consanguineous families. It should be kept in mind that in regions where the prevalence of consanguineous marriages is high, rare variants may cluster among members of the same family.

Ethics

Ethics Committee Approval: The study was performed in accordance with the rules of Declaration of Helsinki and approved by the Institutional Ethics Committee of University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital (no: 69, date: 21.04.2022).

Informed Consent: Informed consent was obtained from all patients included in the study.

Authorship Contributions

Concept: Ruken Yıldırım, Şervan Özalkak, Ayça Aykut, Nevzat Yılmaz, Design: Ruken Yıldırım, Edip Ünal, Şervan Özalkak, Ayça Aykut, Data Collection or Processing: Ruken Yıldırım, Edip Ünal, Şervan Özalkak, Analysis or Interpretation: Ruken Yıldırım, Literature Search: Ruken Yıldırım, Şervan Özalkak, Nevzat Yılmaz, Writing: Ruken Yıldırım, Edip Ünal, Şervan Özalkak.

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References

1. Mendez HM, Opitz JM. Noonan syndrome: a review. Am J Med Genet 1985;21:493-506.
2. Goldmuntz E, Crenshaw ML, Lin AE. Genetic aspects of congenital heart defects. In: Allen HD, Driscoll DJ, Shaddy RE, Feltes TF, (eds). Moss & Adams' Heart Disease in ınfants, Children, and Adolescents ıncluding the Fetus and Young Adult. 8th ed. Philadelphia, Lippincott Williams & Wilkins, 2013;617-644.
3. Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature 1982;297:474-478.
4. Tajan M, Paccoud R, Branka S, Edouard T, Yart A. The RASopathy Family: Consequences of Germline Activation of the RAS/MAPK Pathway. Endocr Rev 2018;39:676-700.
5. Motta M, Sagi-Dain L, Krumbach OHF, Hahn A, Peleg A, German A, Lissewski C, Coppola S, Pantaleoni F, Kocherscheid L, Altmüller F, Schanze D, Logeswaran T, Chahrokh-Zadeh S, Munzig A, Nakhaei-Rad S, Cavé H, Ahmadian MR, Tartaglia M, Zenker M. Activating MRAS mutations cause Noonan syndrome associated with hypertrophic cardiomyopathy. Hum Mol Genet 2020;29:1772-1783.
6. Rohrer T. Noonan syndrome: introduction and basic clinical features. Horm Res 2009;72(Suppl 2):3-7. Epub 2009 Dec 22
7. Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME, Roberts AE, Robinson W, Takemoto CM, Noonan JA. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics 2010;126:746-759. Epub 2010 Sep 27
8. Tartaglia M, Gelb BD, Zenker M. Noonan syndrome and clinically related disorders. Best Pract Res Clin Endocrinol Metab 2011;25:161-179.
9. 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.
10. Mendelian Inheritance in Man, oMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD). Last Accessed Date: 29.04.2022. Available from: https://omim.org/
11. Bowling KM, Thompson ML, Finnila CR, Hiatt SM, Latner DR, Amaral MD, Lawlor JMJ, East KM, Cochran ME, Greve V, Kelley WV, Gray DE, Felker SA, Meddaugh H, Cannon A, Luedecke A, Jackson KE, Hendon LG, Janani HM, Johnston M, Merin LA, Deans SL, Tuura C, Williams H, Laborde K, Neu MB, Patrick-Esteve J, Hurst ACE, Kandasamy J, Carlo W, Brothers KB, Kirmse BM, Savich R, Superneau D, Spedale SB, Knight SJ, Barsh GS, Korf BR, Cooper GM. Genome sequencing as a first-line diagnostic test for hospitalized infants. Genet Med 2022;24:851-861. Epub 2021 Nov 27
12. Bonetti M, Rodriguez-Martinez V, Paardekooper Overman J, Overvoorde J, van Eekelen M, Jopling C, Hertog Jd. Distinct and overlapping functions of ptpn11 genes in Zebrafish development. PLoS One 2014;9:e94884.
13. Ranke MB, Heidemann P, Knupfer C, Enders H, Schmaltz AA, Bierich JR. Noonan syndrome: growth and clinical manifestations in 144 cases. Eur J Pediatr 1988;148:220-227.
14. Yart Yart A, Edouard T. Noonan syndrome: an update on growth and development. Curr Opin Endocrinol Diabetes Obes 2018;25:67-73.
15. Rodríguez F, Gaete X, Cassorla F. Etiology and Treatment of Growth Delay in Noonan Syndrome. Front Endocrinol (Lausanne) 2021;12:691240.
16. Noonan JA, Kappelgaard AM. The efficacy and safety of growth hormone therapy in children with noonan syndrome: a review of the evidence. Horm Res Paediatr 2015;83:157-166. Epub 2014 Dec 10
17. MacFarlane CE, Brown DC, Johnston LB, Patton MA, Dunger DB, Savage MO, McKenna WJ, Kelnar CJ. Growth hormone therapy and growth in children with Noonan's syndrome: results of 3 years' follow-up. J Clin Endocrinol Metab 2001;86:1953-1956.
18. Şıklar Z, Genens M, Poyrazoğlu Ş, Baş F, Darendeliler F, Bundak R, Aycan Z, Savaş Erdeve Ş, Çetinkaya S, Güven A, Abalı S, Atay Z, Turan S, Kara C, Can Yılmaz G, Akyürek N, Abacı A, Çelmeli G, Sarı E, Bolu S, Korkmaz HA, Şimşek E, Çatlı G, Büyükinan M, Çayır A, Evliyaoğlu O, İşgüven P, Özgen T, Hatipoğlu N, Elhan AH, Berberoğlu M. The Growth Characteristics of Patients with Noonan Syndrome: Results of Three Years of Growth Hormone Treatment: A Nationwide Multicenter Study. J Clin Res Pediatr Endocrinol 2016;8:305-312. Epub 2016 Apr 29
19. Croonen EA, Draaisma JMT, van der Burgt I, Roeleveld N, Noordam C. First-year growth in childrenwith Noonan syndrome: Associated with feeding problems? Am J Med Genet A 2018;176:951-958.

20. Jongmans MC, van der Burgt I, Hoogerbrugge PM, Noordam K, Yntema HG, Nillesen WM, Kuiper RP, Ligtenberg MJ, van Kessel AG, van Krieken JH, Kiemeney LA, Hoogerbrugge N. Cancer risk in patients with Noonan syndrome carrying a PTPN11 mutation. Eur J Hum Genet 2011;19:870-874. Epub 2011 Mar 16
21. Cornwall JW, Green RS, Nielsen JC, Gelb BD. Frequency of aortic dilation in Noonan syndrome. Am J Cardiol 2014;113:368-371.
22. Bell JM, Considine EM, McCallen LM, Chatfield KC. The Prevalence of Noonan Spectrum Disorders in Pediatric Patients with Pulmonary Valve Stenosis. J Pediatr 2021;234:134-141. Epub 2021 Mar 29
23. Christou Christou EE, Zafeiropoulos P, Rallis D, Baltogianni M, Asproudis C, Stefaniotou M, Giapros V, Asproudis I. A Narrative Review of the Ocular Manifestations in Noonan Syndrome. Semin Ophthalmol 2022;37:215-221. Epub 2021 Jul 19.
24. Marin Lda R, da Silva FT, de Sá LC, Brasil AS, Pereira A, Furquim IM, Kim CA, Bertola DR. Ocular manifestations of Noonan syndrome. Ophthalmic Genet 2012;33:1-5. Epub 2011 Aug 4
25. van Trier DC, van Nierop J, Draaisma JMT, van der Burgt I, Kunst H, Croonen EA, Admiraal RJC. External ear anomalies and hearing impairment in Noonan Syndrome. Int J Pediatr Otorhinolaryngol 2015;79:874-878. Epub 2015 Apr 1
26. Fowlkes JL, Thrailkill KM, Bunn RC. RASopathies: The musculoskeletal consequences and their etiology and pathogenesis. Bone 2021;152:116060. Epub 2021 Jun 16
27. Cesarini L, Alfieri P, Pantaleoni F, Vasta I, Cerutti M, Petrangeli V, Mariotti P, Leoni C, Ricci D, Vicari S, Selicorni A, Tartaglia M, Mercuri E, Zampino G. Cognitive profile of disorders associated with dysregulation of the RAS/MAPK signaling cascade. Am J Med Genet A 2009;149A:140-146.
28. van der Burgt I, Thoonen G, Roosenboom N, Assman-Hulsmans C, Gabreels F, Otten B, Brunner HG. Patterns of cognitive functioning in school-aged children with Noonan syndrome associated with variability in phenotypic expression. J Pediatr 1999;135:707-713.
29. Johnson EM, Ishak AD, Naylor PE, Stevenson DA, Reiss AL, Green T. PTPN11 Gain-of-Function Mutations Affect the Developing Human Brain, Memory, and Attention. Cereb Cortex 2019;29:2915-2923.
30. Sharland M, Burch M, McKenna WM, Paton MA. A clinical study of Noonan syndrome. Arch Dis Child 1992;67:178-183.
31. Roberts AE. Noonan Syndrome Summary. 2022;1-39. Last Accessed Date: 29.10.2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1124/pdf/Bookshelf_NBK1124.pdf
32. Briggs BJ, Dickerman JD. Bleeding disorders in Noonan syndrome. Pediatr Blood Cancer 2012;58:167-172. Epub 2011 Oct 19

ORIGINAL ARTICLE

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Effect of Adrenocorticotropic Hormone Stimulation on Ischemia-modified Albumin Levels *in vivo*

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What is already known on this topic?

Ischemia-modified albumin (IMA) levels are positively correlated with reactive oxygen species (ROS). It is known that cortisol leads to decreased ROS production. The effect of adrenocorticotropic hormone (ACTH) stimulation on IMA levels is not known.

What this study adds?

We found that standard-dose ACTH stimulation rapidly reduces levels of IMA *in vivo*. It is possible that the relationship between ACTH and IMA is dose-dependent and ACTH has a direct effect on IMA.

Abstract

Objective: Ischemia-modified albumin (IMA) formation is associated with increased reactive oxygen species (ROS) production, while increased cortisol leads to decreased ROS levels. We aimed to evaluate the effect of adrenocorticotropic hormone (ACTH) stimulation on IMA levels and whether the effect was dose-dependent or not.

Methods: A total of 99 subjects with normal ACTH test results were included in the study. Of these, 80 had standard-dose ACTH test while 19 had low-dose ACTH test. Blood samples were collected to determine cortisol and IMA levels; at minutes 0, 30, and 60 following the standard-dose ACTH test and at minutes 0 and 30 following the low-dose ACTH test.

Results: IMA levels decreased significantly within 30 minutes and the decrease continued up to the sixtieth minute ($p = 0.002$) after standard-dose ACTH stimulation. After ACTH stimulation, a weak negative correlation was found between peak cortisol and IMA levels at the thirtieth minute ($r = 0.233$, $p = 0.02$). There was no significant difference in IMA levels after low-dose ACTH stimulation, despite an increase in cortisol ($p = 0.161$).

Conclusion: IMA levels decreased rapidly after standard-dose ACTH stimulation, while a decrease in IMA levels was not observed after low-dose ACTH stimulation. The lack of decrease in IMA levels after low-dose ACTH stimulation suggests a possible dose-dependent relationship between ACTH and IMA. The moderate increase in cortisol with no reduction in IMA levels after low-dose ACTH stimulation and the weak correlation between peak cortisol and 30-minute IMA levels after standard-dose ACTH stimulation suggest that ACTH may have a direct effect on IMA.

Keywords: Ischemia-modified albumin, ACTH, cortisol, reactive oxygen species

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Introduction

Albumin is synthesized in the liver and contains three homologous domains, each consisting of two subdomains (1,2). Albumin contains Sudlow sites 1 and 2, which play an important role in the transport of hydrophobic molecules, heme binding sites, small ligand binding sites, seven fatty acid binding sites, and four metal binding sites, including the sites A and B, N-terminal site (NTS) and Cys34 (2,3,4). Metal binding sites have different affinity for each metal. NTS has the highest affinity for copper, nickel, and cobalt (3). The NTS of albumin is very susceptible to biochemical alteration and degradation.

Reactive oxygen species (ROS) play a role in regulating signaling pathways but must be kept in limited amounts in the body because ROS cause oxidative damage to DNA, RNA, proteins, and lipids (5). Oxidative stress occurs when ROS production exceeds the capacity of antioxidant mechanisms to neutralize ROS (5). Dipeptide cleavage occurs in the NTS of albumin due to increased ROS during oxidative stress (6). The truncated NTS cannot bind metal ions (7). This variant of albumin is called ischemia-modified albumin (IMA). IMA production increases during oxidative stress due to excessive ROS formation (6). Moreover, excessive ROS production has also been shown to play a role in the etiology of various inflammatory diseases through the induction of inflammation (8,9). Therefore, IMA is considered a parameter for the assessmentof both oxidative stress and inflammation.

Cortisol exerts anti-inflammatory effects through genomic and non-genomic pathways, resulting in decreased cytokine and ROS production (10). Adrenocorticotropic hormone (ACTH) has an anti-inflammatory effect through a glucocorticoid-dependent pathway by stimulating endogenous cortisol release. However, the anti-inflammatory effects of ACTH were preserved in adrenalectomized rats (11). ACTH also has a glucocorticoid-independent anti-inflammatory effect by activating melanocortin receptors expressed on immune cells (11). While IMA production increases with excessive ROS formation, ACTH and cortisol cause ROS production to decrease (6,10,11). However, the effect of *in vivo* administration of ACTH, which stimulates endogenous cortisol release, on IMA levels is unknown. The aim of this study was to determine whether ACTH stimulation has an impact on IMA levels and, if so, whether the effect is dependent on the dosage.

Methods

Between February 2021 and February 2022, children who would undergo standard-dose ACTH testing with suspicion of congenital adrenal hyperplasia and children who would undergo low-dose ACTH testing with suspicion of central adrenal insufficiency at a single center were included in the study. Subjects with acute or chronic diseases, active infections, obesity, or drug use were excluded. All participants and/or parents were informed orally and in writing and consented to participate. The study is in accordance with the World Medical Association Declaration of Helsinki and was approved by the University of Health Sciences Turkey, Dr. Sami Ulus Pediatric Health and Disease and Obstetrics and Gynecology Training and Research Hospital Local Ethics Committee (project no: E-21/01-76, date: 21.01.2021).

All subjects were outpatients during the ACTH test. Physical exam and pubertal staging were conducted. For the standard-dose ACTH test, children under two years of age were given 125 mcg of intravenous tetracosactide, while those over two years of age were given 250 mcg. Blood samples were collected during the standard-dose ACTH test at 0, 30, and 60 minutes to measure cortisol and IMA levels, between 08:00 and 09:00 in the morning. Subjects with abnormal standard-dose ACTH stimulation test results were excluded from the study, and 80 subjects who underwent standard dose ACTH tests were included in the study. In the low-dose ACTH test, all subjects were given 1 mcg of tetracosactide. During the low-dose ACTH test, blood samples were taken at 0 and 30 minutes to assess cortisol and IMA levels. Subjects with low-dose ACTH stimulation test results compatible with adrenal insufficiency were excluded from the study. Nineteen subjects who underwent low-dose ACTH tests and had normal results were included in the study. In total, 99 healthy subjects participated in the study.

Chemiluminescent immunoassay was used to analyze serum cortisol concentrations (Siemens Advia XPT). ACTH was analyzed by chemiluminescent immunoassay method (Siemens Immulite 2000 XPi). Blood samples were centrifuged at 3500 rpm for 5 minutes and stored at -80 °C until the IMA test was performed. The albumin cobalt binding test was used to analyze serum IMA concentrations (12). The test procedure is as follows: 50 µL of 0.1% cobalt (II) chloride ($CoCl_2$, $6H_2O$) (Sigma-Aldrich Chemie GmbH, Riedstrasse 2, Steinheim, Germany) was added to 200 µL of subject serum. After mixing, followed by 10 minutes of incubation to allow for albumin cobalt binding, 50 µL 1.5 mg/mL dithiothreitol was added, mixed and incubated for 2 minutes at body temperature. Then 1.0 mL of a 0.9% sodium chloride solution was added in order to reduce the binding capacity. The blank was prepared with distilled water without dithiothreitol. The absorbance of the samples was measured at 470 nm with a spectrophotometer (12) and given as absorbance units.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software, version 17 (IBM Inc., Chicago, IL, USA). Non-parametric tests were used after the normal distribution conformity test. Qualitative data were expressed as numbers/percentage and quantitative data as median (range). The Mann-Whitney U test was used to compare the two groups. To determine the relationship between two continuous variables, the Spearman's correlation coefficient was used to. A statistically significant p value was considered as < 0.05.

Results

The basal ACTH, cortisol, and IMA levels of subjects are given in Table 1. There was no correlation between IMA levels and age of subjects ($p > 0.05$). Basal IMA levels of boys and girls did not differ ($p > 0.05$). More than half (50.5%) of the subjects were pubertal, and the basal and 30th-minute IMA values of the pubertal subjects did not differ from those of the prepubertal subjects ($p = 0.656$ and $p = 0.768$, respectively). There was no correlation found between body mass index (BMI) standard deviation score (SDS) and basal IMA, as well as BMI SDS and 30-minute IMA levels ($r = -0.049$, $p = 0.632$ and $r = -0.129$, $p = 0.204$ respectively). There was no correlation between basal ACTH and IMA as well as basal cortisol and IMA levels ($p > 0.05$).

The results of standard-dose ACTH stimulation: The median age of 80 subjects who underwent standard-dose ACTH test was 5.5 (0.66-17) years. The stimulated cortisol and IMA levels of subjects are given in Table 1. IMA levels decreased significantly within 30 minutes and the decrease continued to 60 minutes after standard-dose ACTH stimulation ($p = 0.002$) (Figure 1). IMA levels at 30 and 60 minutes were similar ($p = 0.773$). There was a positive correlation between basal and 30 as well as basal and 60-minute IMA levels ($r = 0.675$, $p = 0.0001$ and $r = 0.676$, $p = 0.0001$ respectively) (Figure 2). There was a weak negative correlation between peak cortisol and IMA levels 30 minutes after ACTH stimulation ($r = 0.233$, $p = 0.02$) (Figure 3). Basal and stimulated IMA levels did not differ according to gender ($p > 0.05$).

Low-dose ACTH stimulation: Nineteen subjects with a median age of 9.2 (0.1-17.8) years underwent low-dose ACTH test. The stimulated cortisol and IMA levels of subjects are given in Table 1. No significant difference was observed in IMA levels after low-dose ACTH stimulation ($p = 0.161$) (Figure 4). There was no correlation between peak cortisol and IMA levels at the thirtieth minute ($r = 0.103$, $p = 0.667$) (Figure 5). Basal and stimulated IMA levels were not different according to gender ($p > 0.05$).

Comparison of low-dose and standard-dose ACTH stimulation: Basal ACTH and IMA levels were similar in the two groups ($p = 0.353$ and $p = 0.147$, respectively) (Table 1). Although there was a difference between basal cortisol levels, there was no correlation between basal cortisol and basal IMA levels between the two groups ($r = -0.102$, $p = 0.315$). In subjects given standard-dose ACTH stimulation, basal and peak cortisol levels were higher and IMA levels at the thirtieth minute were significantly lower ($p = 0.003$, $p = 0.00001$, and $p = 0.002$ respectively) (Table 1).

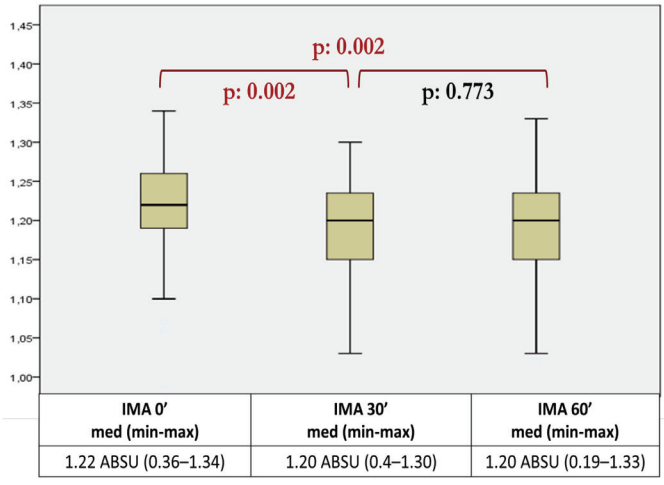

Figure 1. IMA levels after standard-dose ACTH stimulation

IMA: ischemia-modified albumin, ACTH: adrenocorticotropic hormone, Min-max: minimum-maximum, ABSU: absorbance units

Table 1. Comparison of low-dose and standard-dose ACTH stimulation responses

	Low-dose ACTH test			Standard-dose ACTH test			p
	Med	Min	Max	Med	Min	Max	
Basal ACTH pg/mL (pmol/L)	18.4 (4.1)	8.7 (1.9)	43.7 (9.6)	17.7 (3.9)	5 (1.1)	68 (15)	0.353
Basal cortisol mcg/dL (nmol/L)	5.3 (146.3)	1.9 (52.4)	16.8 (463.7)	12 (331.2)	4.1 (113.2)	35.3 (974.3)	0.003
Peak cortisol mcg/dL (nmol/L)	22.5 (621)	18.1 (499.6)	27.7 (750.7)	31.8 (877.7)	19.2 (529.9)	41.9 (1156.4)	0.0001
IMA 0' minute (ABSU)	1.25	0.94	1.29	1.22	0.36	1.34	0.147
IMA 30' minute (ABSU)	1.25	0.96	1.44	1.20	0.40	1.30	**0.002**

ACTH: adrenocorticotropic hormone, IMA: ischemia-modified albumin, Min: minimum, Max: maximum, ABSU: absorbance units

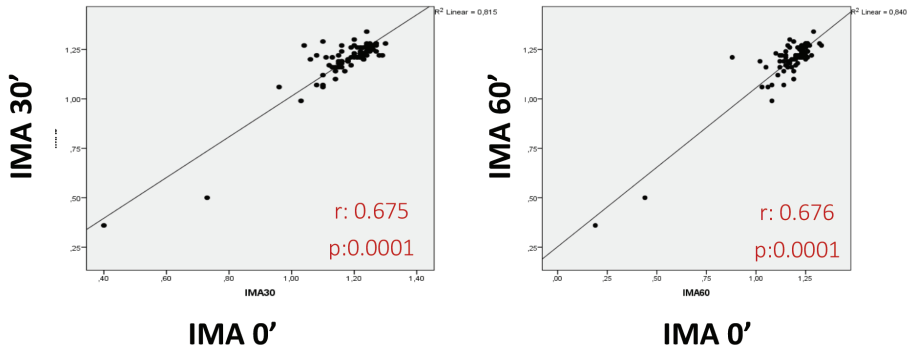

Figure 2. The correlation of basal to 30 and basal to 60 minutes IMA levels after standard-dose ACTH stimulation

IMA: ischemia-modified albumin, ACTH: adrenocorticotropic hormone

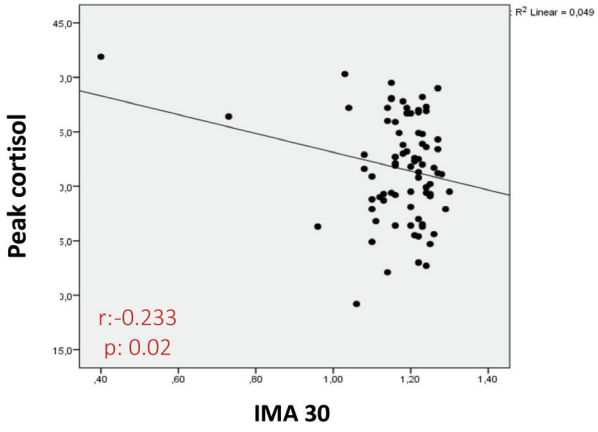

Figure 3. The correlation of peak cortisol and IMA levels at 30 minutes after standard-dose ACTH stimulation

IMA: ischemia-modified albumin, ACTH: adrenocorticotropic hormone

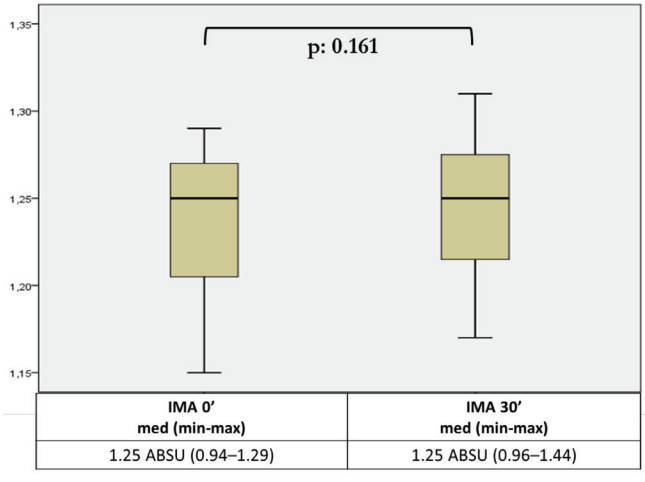

Figure 4. IMA levels after low-dose ACTH stimulation

IMA: ischemia-modified albumin, ACTH: adrenocorticotropic hormone, Min-max: minimum-maximum, ABSU: absorbance units

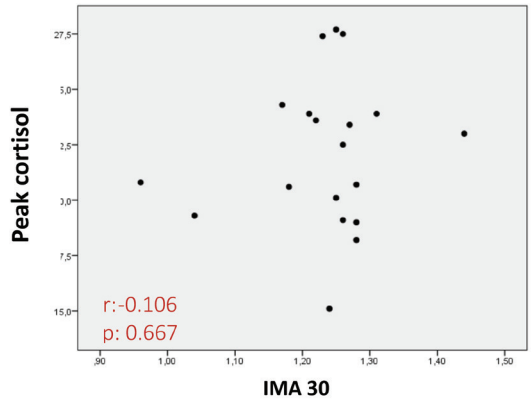

Figure 5. The correlation of peak cortisol and IMA levels at 30 minutes after low-dose ACTH stimulation

IMA: ischemia-modified albumin, ACTH: adrenocorticotropic hormone

Discussion

To the best of our knowledge, this is the first study to evaluate the effect of ACTH stimulation on IMA levels. It is known that increased ROS production during oxidative stress leads to protein oxidation and the production of inflammatory signals, contributing to the development of several chronic diseases (8,9,13,14,15). It has also been shown that IMA formation is associated with increased ROS production and inflammation in oxidative stress and IMA levels rise 6-10 minutes after oxidative stress (6,16,17). However, the alteration of albumin to IMA is reversible, and IMA rapidly returns to its basal level after ischemia (18). In the present study, it was observed that standard dose ACTH stimulation reduced IMA levels *in vivo* at 30 minutes after stimulation. This effect of ACTH on IMA may occur through stimulation of cortisol secretion by ACTH. It has been reported that cortisol strengthens antioxidant defenses by increasing the reduced form of glutathione, thus limiting the production of pro-oxidants such as ROS (19,20). Since cortisol reduces

ROS levels, it is expected to reduce IMA formation. Most glucocorticoid effects are due to the transcriptional effects of the glucocorticoid receptor, which represses the transcription of pro-inflammatory cytokine and chemokine genes (21). However, these mechanisms might not be involved in the more rapid anti-inflammatory effects of glucocorticoids. Studies have shown that glucocorticoids have rapid, non-genomic effects on ROS formation via the generation of intracellular second messengers and signal transduction cascades (22,23,24). In the present study, the rapid decrease in IMA levels after standard-dose ACTH stimulation suggests that cortisol exerts its effect on IMA by a non-genomic pathway. After administering standard-dose ACTH stimulation, we observed a statistically significant but relatively small difference in IMA levels. As it is widely known, high levels of IMA are found in various diseases that are associated with ischemia and oxidative stress. In such cases, ACTH stimulation may lead to a significant decrease in IMA levels. However, it is important to note that our study population comprised healthy individuals. Therefore, the alteration in IMA levels that we observed was relatively low.

IMA levels were also evaluated during a low-dose ACTH test to observe the effect of the ACTH stimulation dose.The lack of a decrease in IMA levels after low-dose ACTH stimulation suggests that a possible dose-dependent relationship between ACTH and IMA. However, the presence of only 19 subjects in the low-dose ACTH test group may have prevented a statistically significant result.

It is known that ACTH is more effective than corticosteroids in treating some inflammatory diseases. Getting et al. (25) demonstrated that local ACTH injection has remarkable anti-inflammatory effects. The same group demonstrated that the anti-inflammatory effects of ACTH were preserved in adrenalectomized rats (25). They also demonstrated that the 4-10th amino acid-containing parts of ACTH cannot stimulate cortisol secretion but can inhibit macrophage activation and neutrophil accumulation in inflammatory exudates (26). In summary, while it is known that ACTH has anti-inflammatory effects other than stimulating cortisol secretion, there is no information that ACTH directly affects ROS and IMA levels. In our study, although standard-dose ACTH stimulation provided a remarkable increase in cortisol and decrease in IMA, there was a weak negative correlation between peak cortisol and IMA levels 30 minutes after ACTH stimulation. There was no significant difference in IMA levels after low-dose ACTH stimulation, despite providing a moderate increase in cortisol. These results indicate that high doses of ACTH may have a direct effect on ROS and IMA levels.

In the present study, in which the effect of ACTH stimulation on IMA levels was evaluated, although the basal IMA and basal ACTH values of the two groups were not different, there was a significant age difference between the two groups. However, we found no correlation between IMA levels and the age of subjects. Moreover, there is no evidence in the literature that suggests IMA changes with age. Since the basal IMA and ACTH level of the two groups is not different we think that age will not be expected to have an effect on the IMA level after ACTH stimulation.

It has been found in some studies that the IMA/albumin ratio should be evaluated alongside IMA levels. A recent review explains that the total plasma albumin concentration should be taken into account, especially in individuals with hypo- or hyperalbuminemia, to avoid misinterpretation of IMA values (27). This is particularly important when examining the level of IMA in other biological fluids such as urine or saliva (27). The study evaluated IMA levels in the blood of healthy participants. It is important to note that since the participants were healthy, it is unlikely that their blood albumin levels would be abnormal.

Study Limitations

The main limitation of our study was the lack of post-stimulation ACTH level measurements. According to the protocol used in our clinic, the amount of standard dose ACTH test tetracosactide is determined according to age, 125 mcg was given to subjects under 2 years of age, and 250 mcg was given to subjects over the age of 2. In contrast, in the low-dose ACTH test, 1 mcg tetracosactide was given to all subjects. Due to the situation, the dosage of ACTH given to the subjects varied based on micrograms per kilogram. It is possible that after administering standard and low doses of ACTH stimulation, the level of ACTH in the blood may have varied among the subjects, which could have influenced our results. However, the lack of correlation between baseline ACTH and IMA suggests that possible individual differences in blood ACTH levels may have a limited effect on the results. The second limitation of our study was that there were only 19 subjects in the low-dose ACTH test group, which may not be sufficient to obtain a statistically significant result.

Conclusion

The effect of ACTH stimulation on IMA was evaluated forthe first timein this study. It was found that standard-dose ACTH stimulation reduced levels of IMA at 30 and 60 minutes after ACTH stimulation *in vivo*, while a decrease is not observed after low-dose ACTH stimulation. The lack of decrease in IMA levels after low-dose ACTH stimulation suggests a

possible dose-dependent relationship between ACTH and IMA. The moderate increase in cortisol with no reduction in IMA levels after low-dose ACTH stimulation and the weak correlation between peak cortisol and thirtieth minute IMA levels after standard-dose ACTH stimulation suggest that ACTH may have some direct effects on IMA levels other than stimulating cortisol secretion. Clarifying functional mechanisms will help understand the pathogenesis of diseases and develop treatments.

Acknowledgement

We would like to thank Fatma Budak for doing the tests and had wonderful cooperation with the children during sampling.

Ethics

Ethics Committee Approval: The study is in accordance with the World Medical Association Declaration of Helsinki and was approved by the University of Health Sciences Turkey, Dr. Sami Ulus Pediatric Health and Disease and Obstetrics and Gynecology Training and Research Hospital Local Ethics Committee (project no: E-21/01-76, date: 21.01.2021).

Informed Consent: All participants and/or parents were informed orally and in writing and consented to participate.

Authorship Contributions

Surgical and Medical Practices: Nursel Muratoğlu Şahin, Senem Esen, Şenay Savaş Erdeve, Semra Çetinkaya, Concept: Nursel Muratoğlu Şahin, Şenay Savaş Erdeve, Salim Neşelioğlu, Özcan Erel, Semra Çetinkaya, Design: Nursel Muratoğlu Şahin, Senem Esen, Data Collection or Processing: Nursel Muratoğlu Şahin, Senem Esen, Şenay Savaş Erdeve, Salim Neşelioğlu, Özcan Erel, Semra Çetinkaya, Analysis or Interpretation: Nursel Muratoğlu Şahin, Şenay Savaş Erdeve, Semra Çetinkaya, Literature Search: Nursel Muratoğlu Şahin, Writing: Nursel Muratoğlu Şahin, Şenay Savaş Erdeve, Semra Çetinkaya.

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References

1. Peters T. "All about Albumin," Biochemistry, Genetics, and Medical Applications, Academic Press, Inc., San Diego, California, 1996.
2. Al-Harthi S, Lachowicz JI, Nowakowski ME, Jaremko M, Jaremko Ł. Towards the functional high-resolution coordination chemistry of blood plasma human serum albumin. J Inorg Biochem 2019;198:110716. Epub 2019 May 20
3. Gaze DC. Biomarkers of cardiac ischemia. Ischemic Heart Disease: Tech Publishing, 2013;91-122.
4. Lexa KW, Dolghih E, Jacobson MP. A structure-based model for predicting serum albumin binding. PLoS One 2014;9:e93323.
5. Durackova Z. Some current insights into oxidative stress. Physiol Res. 2010;59:459-469. Epub 2009 Nov 20
6. Aslan D, Apple FS. Ischemia Modified Albumin Measured by the Albumin Cobalt Binding Test A Clinical and Analytical Review. Lab Med 2004.
7. Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. Eur J Biochem 2001;268:42-47.
8. Sundar IK, Yao H, Rahman I. Oxidative stress and chromatin remodeling in chronic obstructive pulmonary disease and smoking-related diseases. Antioxid Redox Signal 2013;18:1956-1971. Epub 2012 Nov 6
9. Van der Vliet A, Bast A. Effect of oxidative stress on receptors and signal transmission. Chem Biol Interact 1992;85:95-116.
10. Montero-Melendez T. ACTH: The forgotten therapy. Semin Immunol. 2015;27:216-226. Epub 2015 Feb 26
11. Wang W, Guo DY, Lin YJ, Tao YX. Melanocortin Regulation of Inflammation. Front Endocrinol (Lausanne) 2019;10:683.
12. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19:311-315.
13. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 1997;272:20313-20316.
14. Salzano S, Checconi P, Hanschmann EM, Lillig CH, Bowler LD, Chan P, Vaudry D, Mengozzi M, Coppo L, Sacre S, Atkuri KR, Sahaf B, Herzenberg LA, Herzenberg LA, Mullen L, Ghezzi P. Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal. Proc Natl Acad Sci U S A 2014;111:12157-12162. Epub 2014 Aug 5
15. Weseler AR, Bast A. Oxidative stress and vascular function: Implications for pharmacologic treatments. Curr Hypertens Rep 2010;12:154-161.
16. Sinha MK, Vazquez JM, Calvino R, Gaze DC, Collinson PO, Kaski JC. Effects of balloon occlusion during percutaneous coronary intervention on circulating Ischemia Modified Albumin and transmyocardial lactate extraction. Heart 2006;92:1852-1853.
17. Bar-Or D, Winkler JV, Vanbenthuysen K, Harris L, Lau E, Hetzel FW. Reduced albumin-cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty: a preliminary comparison to creatine kinase-MB, myoglobin, and troponin I. Am Heart J 2001;141:985-991.
18. Sinha MK, Vazquez JM, Calvino R, Gaze DC, Collinson PO, Kaski JC. Effects of balloon occlusion during percutaneous coronary intervention on circulating Ischemia Modified Albumin and transmyocardial lactate extraction. Heart 2006;92:1852-1853.
19. Costantini D, Marasco V, Møller AP. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. J Comp Physiol B 2011;181:447-456. Epub 2011 Mar 18
20. Birnie-Gauvin K, Peiman KS, Larsen MH, Aarestrup K, Willmore WG, Cooke SJ. Short-term and long-term effects of transient exogenous cortisol manipulation on oxidative stress in juvenile brown trout. J Exp Biol 2017;220:1693-1700. Epub 2017 Feb 16
21. Smoak KA, Cidlowski JA. Mechanisms of glucocorticoid receptor signaling during inflammation. Mech Ageing Dev 2004;125:697-706.
22. Panettieri RA, Schaafsma D, Amrani Y, Koziol-White C, Ostrom R, Tliba O. Non-genomic Effects of Glucocorticoids: An Updated View. Trends Pharmacol Sci 2019;40:38-49. Epub 2018 Nov 26

23. Croxtall JD, Choudhury Q, Flower RJ. Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. Br J Pharmacol 2000;130:289-298.
24. Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones--a focus on rapid, nongenomic effects. Pharmacol Rev 2000;52:513-556.
25. Getting SJ, Christian HC, Flower RJ, Perretti M. Activation of melanocortin type 3 receptor as a molecular mechanism for adrenocorticotropic hormone efficacy in gouty arthritis. Arthritis Rheum 2002;46:2765-2775.
26. Getting SJ, Gibbs L, Clark AJ, Flower RJ, Perretti M. POMC gene-derived peptides activate melanocortin type 3 receptor on murine macrophages, suppress cytokine release, and inhibit neutrophil migration in acute experimental inflammation. J Immunol 1999;162:7446-7453.
27. Shevtsova A, Gordiienko I, Tkachenko V, Ushakova G. Ischemia-Modified Albumin: Origins and Clinical Implications. Dis Markers. 2021 Jul 19;2021:9945424.

CASE REPORT

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A Novel *KISS1R* Loss-of-function Variant in a Chinese Child with Congenital Hypogonadotropic Hypogonadism

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What is already known on this topic?

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder, resulting from impaired production, secretion, or action of gonadotropin-releasing hormone (GnRH). Mutations of the *KISS1R (GPR54)* gene can result in CHH. Minipuberty is a critical period for genital development due to the activation of the GnRH axis in the initial postnatal months.

What this study adds?

A novel compound heterozygous mutation of *KISS1R* causing CHH in a Chinese boy was reported. The report adds to the spectrum of mutations in the KISS1R gene seen in children with CHH. Evaluation of minipuberty in male newborns and infants who present with micropenis, with or without undescended testes, can help in the early diagnosis and possible early treatment of nCHH.

Abstract

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder, resulting from impaired production, secretion, or action of gonadotropin-releasing hormone (GnRH). Variants of the *KISS1R* gene can result in CHH. Herein we describe a Chinese boy with CHH, caused by a novel, compound heterozygous variant in *KISS1R*. A male infant presented to the pediatric urological surgeon at three months of age for micropenis. Laboratory investigations done at this time revealed low levels of serum gonadotropins and testosterone, suggesting a lack of minipuberty. Topical application of dihydrotestosterone gel was recommended, but the parents refused treatment. The child was brought to our hospital at 3.3 years of age for the same complaint. A diagnosis of CHH was considered, and next generation sequencing revealed a compound heterozygous variant including a novel c.182C > A (p.S61*) and a c.418C > T (p.R140C) in *KISS1R*. We describe a novel compound heterozygous variant in the *KISS1R* in a boy with CHH, born to non-consanguineous Chinese parents. This report adds to the spectrum of variants in *KISS1R* seen in children with CHH.

Keywords: Hypogonadotropic hypogonadism, *KISS1R*, minipuberty

Introduction

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder caused by a defect in the production, secretion, or action of gonadotropin-releasing hormone (GnRH), which regulates the reproductive axis. CHH may present with reproductive symptoms, such as cryptorchidism, micropenis, absent or incomplete puberty, infertility, amenorrhea, and a lack of breast development, and with non-reproductive features, such as bimanual synkinesis, abnormal eye movements, agenesis of the corpus callosum, unilateral or bilateral renal agenesis, cleft lip or palate, alteration of digital bones, and daltonism (1). CHH can be broadly divided into two categories; cases resulting from the abnormal embryonic migration of GnRH neurons from the olfactory placode to the forebrain and associated with anosmia/hyposmia [Kallmann syndrome (KS)], and cases characterized by pure neuro-endocrine impairment of GnRH secretion or action, namely normosmic CHH (nCHH). The incidence of CHH is uncertain. KS has an incidence of 1:125,000 in females and 1:30,000 in males, as reported in a recent epidemiological study (2).

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CHH is seldom diagnosed during childhood, a physiologically hypogonadal period. However, minipuberty is a window for early diagnosis of CHH. Minipuberty is a critical period for genital development due to the activation of the GnRH axis in the initial postnatal months. In boys, there is a rise in the serum testosterone (T) and gonadotropin levels, which peak at three months of age and almost approach adult male levels. This is followed by a decline to low prepubertal levels by about six months. Due to impaired activation of the GnRH axis in minipuberty, low serum T and gonadotropin levels can be observed in infants affected by CHH (3).

Variants of *KISS1R* are found in approximately 5 % of patients with nCHH (4). More than 30 different *KISS1R* variants have been reported to date (5). With the widespread use of genetic testing, genetic causes of CHH are increasingly being identified (1). We present a boy with nCHH born to non-consanguineous, Chinese parents, where a compound heterozygous variant was identified on genetic testing. This included a novel c.182C > A (p.S61*) variant, which was predicted to be pathogenic and the variant c.418C > T (p.R140C), a variant of uncertain significance (VUS) in *KISS1R*.

Case Report

The proband, born to non-consanguineous Chinese parents, was noticed to have a small penis since birth. A pediatric urologist was consulted for the same complaint when the infant was three months old. Investigations showed low levels of serum T and gonadotropins-follicle stimulating hormone (FSH) and luteinizing hormone (LH) (T < 0.03 ng/mL, FSH < 1.1 mIU/mL, and LH < 0.11 U/L). The karyotype was 46, XY. An absence of minipuberty was diagnosed and dihydrotestosterone topical gel was recommended for treatment of micropenis. However, the parents refused treatment at this time.

At 3.3 years of age, the boy was referred to our hospital for investigation of micropenis. There was no family history of any reproductive problems or non-reproductive features associated with CHH. The child could perceive smell. On physical examination, he had a micropenis with stretched length of 1.5 cm (< -4 standard deviation) (6). Neither hypospadias nor cryptorchidism was present. The right testicular volume was 1 mL and the left 0.5 mL. There were no dysmorphic features on general examination and neurological examination was normal.

Investigations showed a basal serum T level of < 0.07 ng/mL, LH < 0.1 IU/L and FSH 1.9 IU/L. The GnRH stimulation test showed a peak LH of 3.9 IU/L at 30 mins and an FSH peak of 26.7 IU/L at 60 mins (Table 1). The results of the 3-day human chorionic gonadotropin (hCG) stimulation test are also shown in Table 1. Serum T increased by 5-10 times on stimulation, which is considered a good response. Serum thyroid stimulating hormone (TSH), free thyroxine (FT4), prolactin, adrenocorticotropic hormone (ACTH), morning cortisol, and insulin-like growth factor-1 (IGF-1) levels were all within the normal range.

An ultrasound scan of the scrotum revealed a right testis of 12 x 6 x 8 mm and a left testes of 11 x 5 x 10 mm. Bone age was 2.2 years (10^{th}-25^{th} percentile) assessed by the Tanner-Whitehouse 2 (TW2) method. Magnetic resonance imaging (MRI) of the brain detected multiple abnormal signals, presenting with slightly longer signals on T1 and T2 weighted images, and slightly higher signal on the flair sequence, in the centrum semiovale and lateral ventricles. The upper edge of the pituitary was sunken. The olfactory bulb and olfactory tract were normal.

Whole exome sequencing and Sanger sequencing revealed a compound heterozygous variant in *KISS1R*. Variant c.182C > A (p.S61*) was inherited from the mother while c.418C > T (p.R140C) was inherited from the father (Figure 1). After annotation via InterVar and classification according to the American College of Medical Genetics and Genomics (ACMG) guidelines, the c.182C > A (NM_032551.5) was classified as pathogenic with evidence levels PVS1 + PM2 + PP3, while c.418C > T was classified as VUS with evidence levels PM1 + PM2 + PP3.

A final diagnosis of nCHH was made and treatment with hCG was recommended to induce penile growth. However, the parents again refused treatment. At 4.6 years of age, the boy was again referred to our hospital for the same complaint. At this visit, the parents opted to use dihydrotestosterone topical gel to treat micropenis rather than hCG or intramuscular T. The child started local application of 2.5% dihydrotestosterone topical gel (0.2-0.3 mg/kg/day) and is due for follow-up after three months.

Table 1. GnRH test and hCG stimulation test

	0 min	30 min	60 min	90 min	120 min		Pre-hCG	Day 3 post-hCG
LH (IU/L)	< 0.1	3.9	3.5	2.5	1.9	Testosterone (ng/mL)	< 0.07	2.19
FSH (IU/L)	2.0	21.7	26.7	25.1	23.6	Dihydrotestosterone (pg/mL)	< 50	69.61

GnRH: gonadotropin-releasing hormone, LH: luteinizing hormone, FSH: follicle-stimulating hormone, hCG: human chorionic gonadotropin

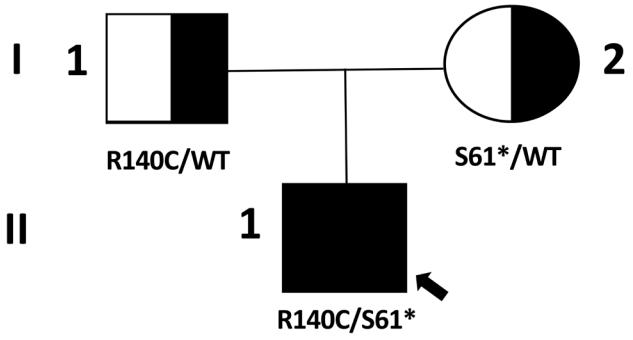

Figure 1. Circles represent females and squares males. Half-shaded symbols indicate unaffected heterozygous and solid symbols affected subjects. The proband, subject II-1 (arrow), was compound heterozygous for the *KISS1R* variants R140C/S61*. The unaffected father (I-1) was heterozygous for the R140C variant and the unaffected mother (I-2) was heterozygous for the S61* variant

Discussion

Interaction of the KISS1R protein and its natural ligand Kisspeptin-1 plays a critical role in initiation and development of puberty. Loss-of-function variants of *KISS1R* responsible for nCHH in humans were first reported in 2003 (7). Most of the previous cases of nCHH associated with *KISS1R* were homozygous variants described in adults and teenagers born of consanguineous parents (5).

In this case report, we describe a prepubertal boy with micropenis, a lack of minipuberty and delayed bone age, suggestive of CHH. CHH remains a diagnosis of exclusion (8) and so malnutrition, any chronic disease, or excessive exercise, the functional causes were exluded. Structural causes, such as tumors, apoplexy, surgery, or infiltrative diseases were also ruled out by brain MRI. Combined pituitary hormone deficiency was also excluded by normal levels of TSH, FT4, prolactin, ACTH, morning cortisol and IGF-1. So the diagnosis of CHH was considered. Whole exome sequencing revealed a compound heterozygous variant in the *KISS1R*, including a novel c.182C > A (p.S61*) (pathogenic) variant and a c.418C > T (p.R140C) (VUS) variant. Loss-of-function variants of *KISS1R* are responsible for nCHH in humans. The boy could perceive smell. The MRI also showed normal olfactory bulb and olfactory tract, presumably since *KISS1R* is involved in GnRH neuron activation and not migration. When clinical data and genetic results were considered together, these variants are most likely to explain the nCHH found in the proband.

The proband was the only child of his parents. He presented with micropenis. Patients with kisspeptin receptor insufficiency may manifest with either complete or partial gonadotropic deficiency. Micropenis in these patients is due to fetal T deficiency resulting from absence of testicular stimulation by gonadotropins during the second and third trimesters of pregnancy (9). Hormonal evaluation revealed low gonadotropins and T concentrations at three months of age, supporting a diagnosis of a lack of minipuberty. Minipuberty is the transient, sex-specific activation of the hypothalamic-pituitary-gonadal axis during the first six months of life in boys (10). A lack of minipuberty in infancy provides a valuable opportunity for the diagnosis of CHH, since childhood is generally a period of physiological hypogonadism (8).

A compound heterozygous variant was found in *KISS1R* in the presented case. The first variant was a nonsense variant, which cause an early termination for translation and was classified as pathogenic according to the ACMG guideline. The second variant was a missense variant, its frequency was extremely low in gnomAD database (0.000006571 for whole database and 0.0001924 for East Asian subgroup of gnomAD database), and relative higher (0.1445%) in the South Asia subgroup of the GenomAsia database (1 allele count in a total of 692). The frequency difference between GenomAsia and gnomAD maybe due to the total cohort number: gnomAD (n = 5198) compared to GenomAsia (n = 692). Other evaluation results included: in cohort > 1000 a pathogenic variant was detected at the trans position in autosomal recessive disease; multiple protein prediction software predicted that the variation was deleterious; and genotype was correlated with phenotype so the variant was classified as VUS.

However, no functional studies were performed to demonstrate the pathogenicity of these variants. Our patient will need long-term follow-up to observe the efficacy of dihydrotestosterone topical gel. Multiple abnormal signals in the centrum semiovale and lateral ventricles, and a sunken upper edge of the pituitary were observed in MRI of the brain, which are not explained at present.

Conclusion

In conclusion, we report a novel compound heterozygous variant of *KISS1R* causing nCHH in a Chinese boy. Evaluation of minipuberty in male newborns and infants who present with micropenis, with or without undescended testes, can help in the early diagnosis and possible early treatment of nCHH. Genetic testing is also recommended to assist the diagnosis of CHH and to provide genetic counseling to the family. Our case adds to the increasing evidence concerning the spectrum of CHH caused by variants in *KISS1R*.

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Ethics

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices – Concept – Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Peng Zhou, Jin Wu.

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References

1. Oleari R, Massa V, Cariboni A, Lettieri A. The Differential Roles for Neurodevelopmental and Neuroendocrine Genes in Shaping GnRH Neuron Physiology and Deficiency. Int J Mol Sci 2021;22:9425.
2. 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.
3. Swee DS, Quinton R. Congenital Hypogonadotrophic Hypogonadism: Minipuberty and the Case for Neonatal Diagnosis. Front Endocrinol (Lausanne) 2019;10:97.
4. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 2009;5:569-576. Epub 2009 Aug 25
5. Alzahrani AJ, Ahmad A, Alhazmi T, Ahmad L. An Isolated Hypogonadotropic Hypogonadism due to a L102P Inactivating Mutation of KISS1R/GPR54 in a Large Family. Case Rep Pediatr 2019;2019:3814525.
6. Schonfeld WA, Beebe GW. Normal growth and variation in the male genitalia from birth to maturity. J Urology 1942;48:759-777.
7. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003;100:10972-10976. Epub 2003 Aug 27
8. Grinspon RP, Freire AV, Rey RA. Hypogonadism in Pediatric Health: Adult Medicine Concepts Fail. Trends Endocrinol Metab 2019;30:879-890. Epub 2019 Aug 27
9. 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.
10. 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.

CASE REPORT

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Identification of a Novel *CYP11B2* Variant in a Family with Varying Degrees of Aldosterone Synthase Deficiency

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What is already known on this topic?

Isolated aldosterone synthase deficiency is a rare autosomal recessive disorder caused by pathogenic variants in *CYP11B2*, resulting in impaired aldosterone synthesis. To date, over forty different pathogenic *CYP11B2* variants have been reported, most of which are missense and nonsense variants that greatly compromise enzymatic activity.

What this study adds?

We report a novel variably pathogenic homozygous variant in the *CYP11B2* gene Chr8:NM_000498.3:c.400G>A p.(Gly134Arg). Interestingly, this homozygous variant led to different clinical phenotypes in two affected relatives. Our study shows that clinicians and geneticists should be alert to the potential pitfalls of *CYP11B2* sequencing due to its homology to *CYP11B1*.

Abstract

Isolated aldosterone synthase deficiency is a rare autosomal recessive disorder caused by pathogenic variants in *CYP11B2,* resulting in impaired aldosterone synthesis. We report on a neonate with isolated aldosterone synthase deficiency caused by a novel homozygous *CYP11B2* variant Chr8:NM_000498.3:c.400G>A p.(Gly134Arg). The patient presented shortly after birth with severe signs of aldosterone deficiency. Interestingly, segregation analysis revealed that the patient's asymptomatic father was also homozygous for the *CYP11B2* variant. Biochemical evaluation of the father indicated subclinical enzyme impairment, characterized by elevated aldosterone precursors. Apparently, this homozygous variant led to different clinical phenotypes in two affected relatives. In this manuscript we elaborate on the biochemical and genetic work-up performed and describe potential pitfalls in *CYP11B2* sequencing due to its homology to *CYP11B1*.

Keywords: Aldosterone synthase, mineralocorticoid, CYB11B2, hypoaldosteronism

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Introduction

Isolated aldosterone synthase deficiency is a rare autosomal recessive disorder caused by pathogenic variants in *CYP11B2* causing impaired aldosterone synthesis. The resulting hyperreninemic hypoaldosteronism leads to a potentially fatal condition, characterized by failure to thrive, dehydration, hyponatremia, hyperkalemia and mild metabolic acidosis (1,2).

We report on a neonate with isolated aldosterone synthase deficiency caused by a novel homozygous *CYP11B2* variant. The proband presented shortly after birth with severe signs of aldosterone deficiency. Segregation analysis revealed that the proband's father harbored the same homozygous *CYP11B2* variant. Although the proband's father appeared asymptomatic, biochemical evaluation showed low aldosterone levels with increased mineralocorticoid precursors suggesting subclinical enzyme impairment. This case complements other reports describing the lack of a clear genotype-phenotype correlation in *CYP11B2* mutations.

Furthermore, this report addresses the encountered pitfalls of *CYP11B2* variant analysis due to its extensive homology with *CYP11B1*, which may delay the genetic diagnosis.

Subjects

A family of Pakistani origin was studied. They were unrelated to previously reported families with pathogenic variants in *CYP11B2*. Written informed consent was obtained from all participants or their legal guardians. All procedures were performed in accordance with the Declaration of Helsinki.

Biochemical Analysis

Steroid hormones were measured in serum using isotope dilution liquid chromatography coupled to tandem mass spectrometry (ID-LC-MS/MS). The steroid profile consisted of cortisol, 17-OH-progesterone (17-OHP), 11-deoxycortisol (Compound S), 11-deoxycorticosterone (11-DOC) and corticosterone (3). Limit of quantitation (LOQ) for these steroids were 1 nmol/L for cortisol and 17-OHP), 0.4 nmol/L for Compound S, 0.1 nmol/L for 11-DOC and 0.5 nmol/L for corticosterone.

Aldosterone was measured in serum using ID-LC-MS/MS (4). The LOQ was 0.03 nmol/L. Plasma renin activity (PRA) was determined in EDTA plasma by measuring the production of angiotensin 1 from angiotensinogen over time using an in-house radio-immunoassay (5). The LOQ was 1 µg angiotensin 1/L/h. Further biochemical analyses of sodium, potassium, chloride, glucose and creatinine were measured on the ISE and C502 module of the Cobas 8000 (Roche). Blood gas parameters pH, pCO_2, bicarbonate and base excess were determined in capillary samples using a RapidPoint 500e (Siemens).

Genetic Analysis

Genomic DNA was extracted from EDTA blood of the patient, his sibling and their parents by standard methods. The coding region and exon–intron boundaries of the *CYP11B2* gene (GenBank: NM_000498.9) were amplified from genomic DNA. Sanger sequencing was performed using the BigDye Terminator Sequencing Kit (Thermo Scientific, Thermo Fisher Scientific, Waltham, USA) and ABI 3730 XL (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA). Coding sequences and flanking intron sequences were analysed with SequencePilot (SeqPatient, JSI Medical Systems GmbH, Ettenheim, Germany). PCR and sequencing primers are available on request.

In silico Analysis

A number of pathogenicity prediction methods were used for *in silico* analysis of variant pathogenicity, including Align GVGD (6), CADD (7), PolyPhen-2 HumVar (8), SIFT (9) and MutationTaster (10). The DUET and DynaMut servers were used to evaluate protein stability upon mutation (11,12). The effects of residue substitution on *CYP11B2* structures, interatomic interactions and vibrational entropy energy were inspected in PyMOL using crystal structure model 4dvq of *CYP11B2* in complex with deoxycorticosterone (13).

Case Report

The proband was the second child of consanguineous, healthy parents of Pakistani origin. He was born at term after an uncomplicated pregnancy and was large for gestational age (LGA) with a birth weight of 3880 grams (91st percentile). There were no congenital anomalies. Because of LGA, glucose levels were measured, revealing hypoglycemia in the first hours after birth, which was treated with intravenous glucose. After two days, he was discharged from hospital on a normal newborn feeding formula, without any medication or supplements.

At the age of three weeks, the proband was seen at our outpatient clinic and was subsequently readmitted because of persistent vomiting and failure to thrive. At that time his weight was more than ten percent below birth weight (3415 grams). Physical examination revealed dehydration without apparent signs of infection.

Laboratory evaluation (Table 1A) showed hyponatremia, hyperkalemia and metabolic acidosis. Initially, primary adrenal insufficiency was suspected and after performing

Table 1. Laboratory results of the index patient (A) at presentation (age three weeks) and of the patient's asymptomatic father (B)

A. Results of the index patient		
Measurement		**Reference interval***
Sodium	124	133-142 mmol/L
Potassium	7.6	4.0-6.0 mmol/L
Chloride	89	98-113 mmol/L
pH (capillary)	7.3	7.32-7.43
pCO_2	5.6	3.5-5.5 kPa
Bicarbonate	20.1	23-29 mmol/L
Base excess	-6.1	-2-10 mmol/L
Glucose	4.4	2.8-4.5 mmol/L
Creatinine	42	35-80 µmol/L
Aldosterone	0.04**	< 0.54 nmol/L
Plasma renin activity	66	< 7.5 µg angiotensin 1/L/h
Cortisol	186	250-650 nmol/L
B. Results of the patient's father		
Measurement		Reference interval
Sodium	140	–135-145 mmol/L
Potassium	4.2	3.5-4.5 mmol/L
Aldosterone	< 0.03***	< 0.54 nmol/L
Plasma renin activity	6.1	< 7.5 µg angiotensin 1/L/U
Cortisol	284	250-650 nmol/L
17-OH progesterone	1.3	< 7.6 nmol/L
11-deoxycortisol	1.4	< 3.2 nmol/L
11-deoxycorticosterone	3.8	< 0.82 nmol/L
Corticosterone	27.7	1.8-56 nmol/L

*All reference intervals are age-specific reference intervals, except for aldosterone and plasma renin activity for which adult reference ranges are depicted. **Aldosterone LOQ: 0.03. ***The aldosterone concentration was below LOQ.
LOQ: limit of quantitation

a cosyntropin stimulation test, the patient was started on hydrocortisone 50 mg/m^2/day in four daily doses and intravenous isotonic (0.9%) saline. Basal cortisol and aldosterone levels were low-normal and very low (just above LOQ), respectively. PRA was increased and renal abnormalities were excluded by a renal ultrasound. The cosyntropin stimulation test (36 microgram/kg bodyweight) showed an appropriate rise of post-stimulation cortisol levels, ruling out glucocorticoid deficiency (Table 2). 17-OHP and 11-deoxycortisol levels were slightly increased at baseline, but only a marginal rise was seen following cosyntropin stimulation (Table 2). After ruling out glucocorticoid deficiency, isolated mineralocorticoid deficiency was suspected. Hydrocortisone was stopped after two days and treatment with fludrocortisone 62.5 µg twice daily was started. Precursors of the aldosterone synthesis pathway were measured in pre-treatment samples. Clearly elevated levels of 11-DOC and corticosterone (Table 2) were found, confirming the biochemical diagnosis of aldosterone synthase deficiency.

After initiating therapy, rapid clinical and biochemical recovery was noted. Intravenous saline treatment was changed to an oral hypertonic saline solution at the age of five weeks. Because of persistent subnormal sodium levels, the fludrocortisone dose was increased to 100 µg twice daily. With this treatment regimen, the patient was growing and developing well with normal sodium, potassium and PRA. At the age of one year, the patient was challenged by reducing the fludrocortisone dose to 62.5 µg twice daily. This resulted in recurrence of salt-loss necessitating reinstitution of the original fludrocortisone dose, confirming the persistence of clinically relevant hypoaldosteronism.

A heterozygous variant of unknown significance was detected in exon 3 of the *CYP11B2* gene after sequencing the coding region and the splice sites (NM_000498.3): Chr8:NM_000498.3:c.400G > A p.(Gly134Arg). As heterozygous variants are usually asymptomatic/subclinical, the heterozygous variant seemed an insufficient explanation for the observed phenotype of our patient.

To find an alternative explanation for the observed phenotype and because of the parental consanguinity, our first step was to search for interesting regions of homozygosity (ROHs). We performed genome wide array

Table 2. Results of a cosyntropin stimulation test in a three-week-old boy suspected of glucocorticoid and/or mineralocorticoid deficiency

Measurement	**T = 0 (unstimulated)**	**T = 30 min**	**T = 60 min**	**Reference interval (unstimulated)**
Cortisol	602	761	873	250-650 nmol/L
17-OH progesterone	5.6	9.7	11.3	< 3.2 nmol/L
11-deoxycortisol (Compound S)	10.9	12.2	13.8	< 3.2 nmol/L
11-deoxycorticosterone (11-DOC)	10.2	14.1	17.4	< 0.82 nmol/L
Corticosterone	353	547	639	1.8-56 nmol/L

All steroids were measured with LC-MS/MS. Reference intervals are intervals for the unstimulated state in adults, except for 17-OHP (age adjusted).
17-OHP: 17-OH progesterone

analysis using the CytoScan XON array platforms (Thermo Fisher Scientific, Inc., Waltham, MA, USA) on DNA from the index patient following the manufacturer's instructions. We did not identify a copy number loss or gain, but detected multiple, relatively large ROHs in the exome data (196 Mb of the autosomal genome (~6.5%), which is in agreement with the indicated parental consanguinity. The *CYP11B2* gene lies within a ROH of about 7 Mb. Due to the discrepancy between the results, another set of primers for exon 3 of the *CYP11B2* gene was designed, which led to the discovery that the c.400G>A variant was in fact present in a homozygous state. This is consistent with the genetic diagnosis of isolated aldosterone synthase deficiency due to a homozygous missense variant in *CYP11B2* (c.400G>A). The initially used primers had also recognized and amplified the highly homologous region of the *CYB11B1* gene, leading to false heterozygous detection of the variant.

The detected variant has not been reported in the literature before and is present in a very low allele frequency (0.0012%) in the genome Aggregation Database (gnomAD, v.2.1.1) (http://gnomad.broadinstitute.org). Segregation analysis was performed in the asymptomatic parents and sibling. The proband's clinically unaffected mother and sister appeared to be heterozygous carriers of the novel *CYB11B2* variant but the patient's father harbored the variant in a homozygous state. This was an unexpected finding, as the father appeared clinically asymptomatic whilst on a regular Western diet, and had not experienced any symptoms during infancy or childhood. This result also argued against the pathogenicity of the detected *CYP11B2* variant, and for that reason an endocrine work-up of the father was performed. On physical examination, the father had normal height and his vital signs were unremarkable. Biochemical evaluation showed normal electrolytes and PRA, but low aldosterone and clearly increased 11-DOC (an aldosterone precursor) concentrations (Table 1B), suggesting a mild (subclinical) aldosterone synthase deficiency.

To investigate the variant's pathogenicity, *in silico* analyses were performed. Gly at position 134 is highly conserved across species (Figure 1) and appears to be an exposed residue in the Cytochrome P450 domain of *CYP11B2*. The variant is predicted to be deleterious by several pathogenicity prediction methods (Table 3). Results of protein structural analysis indicate that the surface of the mutated *CYP11B2* is changed (Figure 2). These changes may negatively influence protein-substrate dynamics. Protein stability analysis suggests decreased protein stability, which may lead to a misfolded protein (Table 3), perturbing substrate access to the active site or substrate binding and could also lead to increased degradation. These findings suggest that Gly at position 134 is a functional residue.

Discussion

Aldosterone is the principal mineralocorticoid in humans. Its main actions are to regulate intravascular volume

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P19099_C11B2_HUMAN      CRMILEPWVAYRQHRGHKCGVFLLNGPEWRFNRLRLNPDVLS 150
MUTANT_HUMAN            CRMILEPWVAYRQHRGHKCGVFLLNRPEWRFNRLRLNPDVLS 150
P15538_C11B1_HUMAN      HRMSLEPWVAYRQHRGHKCGVFLLNGPEWRFNRLRLNPEVLS 150
P15539_C11B2_MOUSE      RRMHLEPWVAHRELRGLRRGVFLLNGPEWRLNRLRLNRNVLS 150
P30099_C11B2_RAT        RRMHLEPWVAHRELRGLRRGVFLLNGAEWRFNRLKLNPNVLS 160
Q0P493_Q0P493_DANRE     RRMTLQPWATHRETRRHSKGVFLKNGTEWRADRLLLNREVMV 160
W0FJN1_W0FJN1_PIG       QRMFLEPWLAYRQLRGHKCGVFLLNGPTWRLDRLQLNPGVLS 150
F1PNC7_F1PNC7_CANLF     WRPPLDPWLAYRQHRGHKCGVFLLNGPEWRLNRLKLNPDVLS 150
                         *  *:** ::*: *    **** **  ** :** **  *:
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Figure 1. Sequence alignment. The Gly at position 134 of the *CYP11B2* gene is highly conserved across species suggesting structural and/or functional importance

Table 3. Pathogenicity score of the p.(Gly134Arg) variant in several pathogenicity prediction programs

Prediction method	Score
Align GVGD	C65 (very likely to interfere with function)
CADD	24.3 (top 1% deleterious variants)
MutationTaster	1 (disease causing)
PolyPhen-2 (HumVar)	1.000 (probably damaging)
SIFT	0 (deleterious)
DUET	ΔΔG: -1.26 kcal/mol (destabilizing)
DynaMut	ΔΔG: -0.59 kcal/mol (destabilizing)

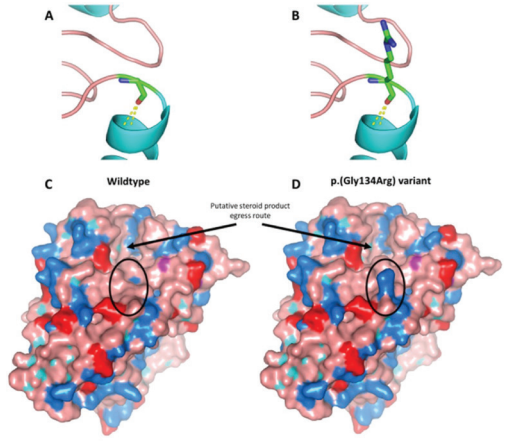

Figure 2. *In silico* protein modelling of *CYP11B2* p.(Gly134Arg)

Crystal structure model 4DVQ of human aldosterone synthase (*CYP11B2*) in complex with deoxycorticosterone (13) was used to study interatomic interactions upon mutation, employing PyMOL. Positively charged amino acids are blue, negatively charged amino acids are red. p.(Gly134Arg) substitution (panels A and B, the affected amino acid is given in green) did not result in steric clashes or changes to hydrogen bridges (yellow dashed lines). However, dihedral angles of p.(Gly134Arg) are considerably different to wild-type; the arginine sidechain (black circle, panel D) is pointed outwards, so that the otherwise smooth patch of protein surface has a positively charged appendicle (panels C and D). This may influence protein-substrate dynamics, since it is in close proximity to a putative steroid product egress route (13)

and electrolyte balance. Aldosterone is synthesized from cholesterol in the zona glomerulosa of the adrenal cortex. Aldosterone synthase is a mitochondrial cytochrome P450 enzyme, encoded by the *CYP11B2* gene located on chromosome 8q24.3 and containing nine exons (14). This enzyme is involved in the last three steps of the aldosterone biosynthesis pathway facilitating the 11-hydroxylation of deoxycorticosterone to corticosterone, the subsequent 18-hydroxylation to hydroxycorticosterone, and finally 18-oxidation to aldosterone (15). 18-hydroxycorticosterone can be measured to distinguish between 18-hydroxylase deficiency (type 1) and 18-oxidase deficiency (type 2). We chose not to measure 18-hydroxycorticosterone as this distinction would not have clinical implications.

Aldosterone synthase deficiency biochemically leads to hyperreninemic hypoaldosteronism, characterized by hyponatremia, hyperkalemia, metabolic acidosis, increased renin and low or low-normal aldosterone levels. As demonstrated in the present case, increased levels of mineralocorticoid precursors before the enzymatic block may be found. Clinically, the disease manifests in the neonatal period or early infancy with vomiting, dehydration, and failure to thrive (16,17,18). These symptoms are nonspecific and can easily be mistaken for more common conditions.

To date, more than forty different pathogenic variants in the *CYP11B2* gene causing isolated aldosterone synthase deficiency have been reported, most of which are missense and nonsense variants that greatly compromise enzymatic activity (17,18). In a recent study, the missense variant c.554C > T p.(Thr185Ile) was found, either in a homozygous or compound heterozygous state, in the vast majority of the studied patients (17). To the best of our knowledge, the missense variant c.400G > A p.(Gly134Arg) detected in the proband and his family members has not been described before.

Based on the location of the variant, in close proximity to two earlier described pathogenic variants (17), the known deleterious effect of the variant in the highly homologous *CYP11B1* gene (19), the low frequency in the gnomAD database, the results of prediction programs, the results of protein modeling and the appropriate clinical and biochemical findings in the proband, strongly suggest this variant to be pathogenic, in our opinion.

The proband's father with the same homozygous *CYP11B2* variant appeared asymptomatic, but biochemical analysis revealed low aldosterone levels and an elevated 11-DOC concentration, suggesting impaired enzyme activity. Apparently, this variant is not fully penetrant. Reduced penetrance has previously been reported in members of an extended Palestinian family, harboring a different variant in *CYB11B2* [c.1354G > T; p.(G452W)] (20). In this family, two clinically unaffected mothers were found to be homozygous for the same variant as their affected offspring. Why the severity of enzyme impairment can differ

between people with the same genotype is unclear. Faingelernt et al. (20) speculated that undetermined genetic or epigenetic factors, for example affecting sensitivity to mineralocorticoid action or impairing 11β-hydroxysteroid dehydrogenase type 2 activity, could play a role.

Lastly, we would like to alert clinicians to the pitfalls we encountered trying to obtain a genetic diagnosis. The two 11β-hydroxylase iso-enzymes, 11β-hydroxylase enzyme (P450c11β) and aldosterone synthase enzyme (P450c11AS), are encoded by the 93%-identical *CYP11B1* and *CYP11B2* genes, respectively (21). Both genes are located in band q24.3 of chromosome 8 and are approximately 40 kb apart. Their sequence homology complicates genetic analysis as illustrated by the presented case and highlights the importance of using 100% specific primers for each gene.

There have been several reports of patients with hyperreninemic hypoaldosteronism in whom a genetic diagnosis could not be made (21,22). The present case raises the question whether similar false negative results, caused by the close homology between the two *CYP11B* genes, could have played a role in the inability to obtain a genetic diagnosis in these patients.

Conclusion

Herein, we report the case of a neonate with hyperreninemic hypoaldosteronism due to isolated aldosterone synthase deficiency, caused by a novel, homozygous *CYP11B2* variant. The patient's father was homozygous for the same variant and appeared asymptomatic. However, biochemical evaluation revealed suboptimal aldosterone synthase function, suggesting a phenotypical spectrum based on enzymatic residual activity that can vary within a family. The explanation for this observed lack of a clear genotype-phenotype correlation remains elusive. This case shows that establishing a genetic diagnosis of isolated aldosterone synthase deficiency may be complicated by the homology between *CYP11B2* and *CYP11B1*.

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Ethics

Informed Consent: Written informed consents were obtained from all participants and their legal guardians. All procedures were performed in accordance with the Declaration of Helsinki.

Authorship Contributions

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References

1. Root AW. Disorders of aldosterone synthesis, secretion, and cellular function. Curr Opin Pediatr 2014;26:480-486.
2. White PC. Aldosterone synthase deficiency and related disorders. Mol Cell Endocrinol 2004;217:81-87.
3. Büttler RM, Martens F, Ackermans MT, Davison AS, van Herwaarden AE, Kortz L, Krabbe JG, Lentjes EG, Syme C, Webster R, Blankenstein MA, Heijboer AC. Comparison of eight routine unpublished LC-MS/MS methods for the simultaneous measurement of testosterone and androstenedione in serum. Clin Chim Acta 2016;454:112-118. Epub 2016 Jan 8
4. Bekkach Y, Heijboer AC, Endert E, Ackermans MT. Determination of urinary aldosterone using a plasma aldosterone 2D ID LC-MS/MS method. Bioanalysis 2016;8:1765-1775. Epub 2016 Aug 5
5. Hollemans HJ, van der Meer J, Kloosterziel W. Measurement of renin activity in plasma by a radioimmunoassay for angiotensin I. Clin Chim Acta 1969;24:353-357.
6. Tavtigian SV, Deffenbaugh AM, Yin L, Judkins T, Scholl T, Samollow PB, de Silva D, Zharkikh A, Thomas A. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet 2006;43:295-305. Epub 2005 Jul 13
7. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46:310-315. Epub 2014 Feb 2
8. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248-249.

9. Vaser R, Adusumalli S, Leng SN, Sikic M, Ng PC. SIFT missense predictions for genomes. Nat Protoc 2016;11:1-9. Epub 2015 Dec 3
10. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361-362.
11. Rodrigues CH, Pires DE, Ascher DB. DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. Nucleic Acids Res 2018;46:350-355.
12. Pires DE, Ascher DB, Blundell TL. DUET: a server for predicting effects of mutations on protein stability using an integrated computational approach. Nucleic Acids Res 2014;42:314-319. Epub 2014 May 14
13. Strushkevich N, Gilep AA, Shen L, Arrowsmith CH, Edwards AM, Usanov SA, Park HW. Structural insights into aldosterone synthase substrate specificity and targeted inhibition. Mol Endocrinol 2013;27:315-324. Epub 2013 Jan 15
14. Mornet E, Dupont J, Vitek A, White PC. Characterization of two genes encoding human steroid 11 beta-hydroxylase (P-450(11) beta). J Biol Chem 1989;264:20961-20967.
15. Curnow KM, Tusie-Luna MT, Pascoe L, Natarajan R, Gu JL, Nadler JL, White PC. The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. Mol Endocrinol 1991;5:1513-1522.
16. Turan I, Kotan LD, Tastan M, Gurbuz F, Topaloglu AK, Yuksel B. Molecular genetic studies in a case series of isolated hypoaldosteronism due to biosynthesis defects or aldosterone resistance. Clin Endocrinol (Oxf) 2018;88:799-805. Epub 2018 Apr 18
17. Merakou C, Fylaktou I, Sertedaki A, Dracopoulou M, Voutetakis A, Efthymiadou A, Christoforidis A, Dacou-Voutetakis C, Chrysis D, Kanaka-Gantenbein C. Molecular Analysis of the CYP11B2 Gene in 62 Patients with Hypoaldosteronism Due to Aldosterone Synthase Deficiency. J Clin Endocrinol Metab 2021;106:182-191.
18. Miao H, Yu Z, Lu L, Zhu H, Auchus RJ, Liu J, Jiang J, Pan H, Gong F, Chen S, Lu Z. Analysis of novel heterozygous mutations in the CYP11B2 gene causing congenital aldosterone synthase deficiency and literature review. Steroids 2019;150:108448. Epub 2019 Jul 11
19. Zhou Q, Wang D, Wang C, Zheng B, Liu Q, Zhu Z, Jia Z, Gu W. Clinical and Molecular Analysis of Four Patients With 11β-Hydroxylase Deficiency. Front Pediatr 2020;8:410.
20. Faingelernt Y, Hershkovitz E, Abu-Libdeh B, Abedrabbo A, Abu-Rmaileh Amro S, Zarivach R, Zangen D, Lavi E, Haim A, Parvari R, Abu-Libdeh A. Aldosterone synthase (CYP11B2) deficiency among Palestinian infants: Three novel variants and genetic heterogeneity. Am J Med Genet A 2021;185:1033-1038. Epub 2021 Jan 13
21. Klomchan T, Supornsilchai V, Wacharasindhu S, Shotelersuk V, Sahakitrungruang T. Novel CYP11B2 mutation causing aldosterone synthase (P450c11AS) deficiency. Eur J Pediatr 2012;171:1559-1562. Epub 2012 Jul 17
22. Kayes-Wandover KM, Tannin GM, Shulman D, Peled D, Jones KL, Karaviti L, White PC. Congenital hyperreninemic hypoaldosteronism unlinked to the aldosterone synthase (CYP11B2) gene. J Clin Endocrinol Metab 2001;86:5379-5382.

CASE REPORT

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Early Diagnosis of Wolfram Syndrome by Ophthalmologic Screening in a Patient with Type 1B Diabetes Mellitus: A Case Report

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What is already known on this topic?

Wolfram syndrome (WS) is typically associated with childhood-onset insulin dependent diabetes mellitus (DM) as a first manifestation and progressive optic atrophy. Recently, the *WFS1* variants have been reported to be a major cause of autoantibody-negative type 1 DM (type 1B DM; T1BDM), but there are no clinical screening methods for WS without symptoms other than those of DM. Therefore, patients with WS remains misdiagnosed as T1BDM.

What this study adds?

We report a case of WS diagnosed by ophthalmologic screening before the appearance of visual impairment. The male patient developed T1BDM at the age of 3 years. At the age of 6 years, his endogenous insulin secretion was impaired but was still preserved. Fundus examinaton at that time revealed optic nerve head pallor, and *WFS1* gene analysis confirmed the diagnosis of WS. We propose that patients with T1BDM who have preserved endogenous insulin secretion may be eligible for ophthalmologic screening to detect WS, even if they are younger than suggested for current ophthalmologic screening for detection of diabetic retinopathy.

Abstract

Wolfram syndrome (WS) is a monogenic diabetes caused by variants of the *WFS1* gene. It is characterized by diabetes mellitus (DM) and optic atrophy. Individuals with WS initially present with autoantibody-negative type 1 DM (type 1B DM; T1BDM). The diagnosis is often delayed or misdiagnosed, even after visual impairment becomes apparent. We report a case of WS diagnosed by ophthalmologic screening before the appearance of visual impairment. A 7-year-old male patient developed T1BDM at the age of 3 years. At 6 years of age, his endogenous insulin secretion decreased but was not completely absent, and glycemic control was good with insulin treatment. Fundus examination at that time revealed optic nerve head pallor, and *WFS1* gene analysis confirmed a compound heterozygous variant (c.2483delinsGGA/c.1247T > A). Ophthalmological screening can help in early diagnosis of WS in T1BDM, especially when endogenous insulin secretion is preserved, which would facilitate effective treatment.

Keywords: Wolfram syndrome, type 1B diabetes mellitus, ophthalmologic screening

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Introduction

Wolfram syndrome (WS) is a rare, autosomal recessive genetic, disorder characterized by juvenile-onset diabetes mellitus (DM), optic atrophy (OA), diabetes insipidus (DI), deafness, and neurological complications. Typically, in most cases of WS, OA is detected by subjective symptoms, such as decreased vision, and is diagnosed later in the course of the disease (1). Recently, variants in the *WS* gene, *WFS1*, have been reported to be a major cause of autoantibody-negative type 1 DM (type 1B DM; T1BDM) (2,3). However, it is difficult to clinically diagnose WS unless the patient presents with symptoms other than those of diabetes. Here, we report a case of WS in which the diagnosis was made by ophthalmologic screening and subsequent genetic analysis, before the patient presented with obvious vision loss.

Case Report

The patient was a 7-year-old Japanese boy. He was the third child of healthy, non-consanguineous Japanese parents. His sister, brother, and parents had no signs or symptoms of diabetes or visual impairment. At the age of 3 years, he was diagnosed with DM following presentation with symptoms of polyuria and polydipsia. His laboratory test results at the time of diagnosis were: blood glucose level, 682 mg/dL; hemoglobin A1c (HbA1c) level, 12.2%; serum fasting C-peptide level, 0.30 ng/mL; serum immunoreactive insulin concentration, 0.8 μU/mL. Notably; all four anti-autoantibodies directed against pancreatic islets, including anti-glutamic acid decarboxylase (GAD) antibody, anti-insulin antibody, anti-insulinoma-associated protein-2 (IA-2) antibody and anti-zinc transporter 8 antibody, were negative. These findings led to the diagnosis of T1BDM, for which he was treated with subcutaneous insulin injection therapy. Insulin therapy twice daily was initiated, and at 5 years, his insulin therapy was transitioned to basal-bolus therapy.

At the age of 6 years, the patient was referred to Asahikawa Medical University Hospital for initiation of insulin-pump therapy. On a total daily dose of insulin of 0.46 units/kg/day, his HbA1c level was 6.9% and his serum fasting C-peptide level was 0.24 ng/mL, indicating that endogenous insulin secretion was impaired but still preserved. The islet-specific autoantibodies, anti-GAD antibody, anti-insulin antibody, and anti-IA-2 antibody were all rechecked and all remained negative.

Thus, ophthalmologic screening was performed to determine the cause of his T1BDM. His uncorrected visual acuity was 0.15 in the right eye and 0.2 in the left eye. Ophthalmoscopy revealed OA in both eyes, manifesting as optic disc pallor (Figure 1). Critical flicker frequency, which can detect optic nerve disease (normal, >35 Hz; abnormal, <25 Hz), was abnormal in both eyes; 23.1 Hz in the right eye and 20.8 Hz in the left eye. Magnetic resonance imaging revealed no obvious optic nerve atrophy. No abnormalities were detected on screening for other complications associated with WS, including hearing loss, urinary tract malformations, DI, and psychiatric symptoms.

Mutation Analysis for *WFS1*, *INS*, *KCNJ11* and *ABCC8*

Based on the above clinical findings, WS was suspected and *WFS1* gene analysis was performed. In addition, the *INS*, *KCNJ11*, and *ABCC8* genes were also investigated to exclude early onset monogenic diabetes (4). Blood samples were collected from the patient and his mother, from which genomic DNA was obtained. His father refused to undergo genetic analysis. This study was approved by the Asahikawa Medical University Research Ethics Committee, and informed consent was obtained from the parents. Direct sequencing was undertaken to analyze all exon and exon-intron boundary regions of the *WFS1*, *INS*, *KCNJ11*, and *ABCC8* genes. No variants were identified in the INS, *KCNJ11*, and *ABCC8* genes. We identified two heterozygous variants in exon 8 of the *WFS1* gene. The first variant was NM_006005.3: c.2483delinsGGA, resulting in a frameshift and premature stop codon (p.Ile828Arg fs*35) (Figure 2A). This variant has been reported to cause WS in Japanese patients (5). The second variant was NM_006005.3: c.1247T>A (p.Ile416Asn) (Figure 2B). The allele frequency of this variant is 6.98×10^{-6} in gnomAD and 7.96×10^{-5} in TOPmed, a comprehensive Japanese genetic variation database. *In silico* analysis predicted that this variant was probably damaging (Polyphen-2) or deleterious (PROVEAN). These two variants were confirmed to be in different alleles using the TA cloning method and showed compound heterozygous variants. Based on the American College of Medical Genetics and Genomic standards and guidelines, the

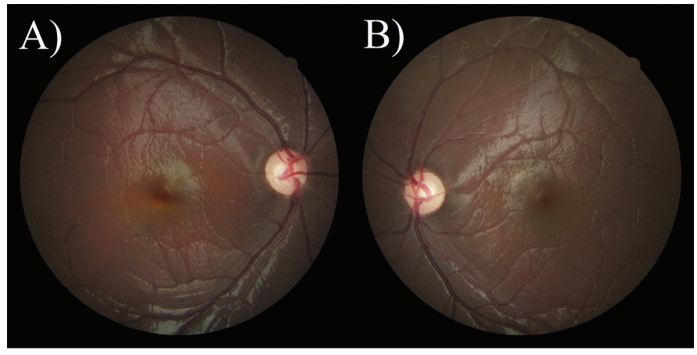

Figure 1. Fundus images of the right (A) and the left (B) eye, revealing optic disc pallor

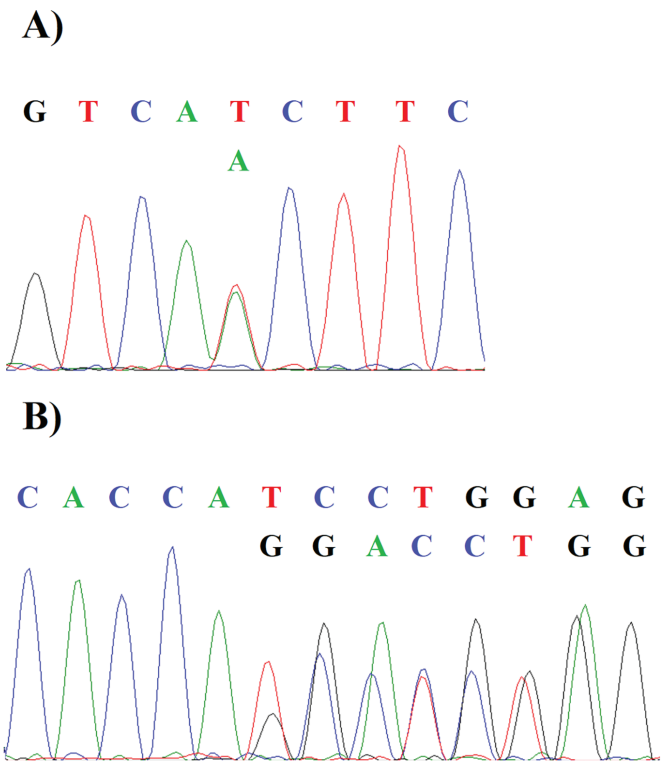

Figure 2. Sequence of the patient's *WFS1* gene. A) Forward sequences of position from 1243 to 1251. T to A single nucleotide change at position 1247 for heterozygosity. B) Forward sequences of position from 2478 to 2490. Single deletion of T and insertion of GGA at position 2483 for heterozygosity

c.2483delinsGGA and c.1247T>A variants were classified as pathogenic and likely pathogenic, respectively. Analysis of the mother, for these *WFS1* variants, showed that she had only the c.2483delinsGGA variant.

Discussion

Herein, we report a case of WS diagnosed with T1BDM at the age of 3 years. The patient was suspected of having WS based on optic nerve head pallor during ophthalmologic screening at the age of 6 years and this was subsequently confirmed by *WFS1* gene analysis.

WS usually presents with DM at a median age of 6 to 8 years, and subsequently manifests with OA at a median age of 11 to 15 years (1,5). In a previous report from Japan, 80% of cases (32 out of 40) followed the typical course (5). Therefore, WS usually remains misdiagnosed as autoantibody-negative DM for a long time (6). These authors, Zmyslowska et al. (6) reported that, although OA was identified based on progressive vision loss over an average period of 4 years after the diagnosis of DM, it took an additional average of 7 years for the patients to be diagnosed with WS. In addition to DM and OA, patients with WS may manifest with a variety of symptoms, such as hearing loss, urinary tract malformations, DI, and psychiatric symptoms (1). Therefore, early diagnosis is important for early management of these complications. Recently, off-label use of a GLP-1 receptor analog for WS showed promising results in preventing disease progression; patients receiving this treatment showed no deterioration of insulin secretion and no significant changes in ophthalmological, neuroradiological, and neurophysiological parameters during follow-up (7). Thus, early detection of WS in patients with DM is important, considering the availability of this promising therapy.

Recently, a comprehensive genetic analysis of T1BDM suggested that *WFS1* may be the major causative gene in T1BDM (2,3). There are no reports of whether patients with *WFS1* variants, detected by comprehensive genetic analysis, showed OA or other complications, except for one who had DM alone (2,3). Therefore, the presented patient may be one of the few cases in which early ophthalmologic screening was indicative of WS, which was confirmed by gene analysis of *WFS1*, before the appearance of visual symptoms, instead of a comprehensive genetic analysis.

The ISPAD Clinical Practice Consensus Guidelines recommend that ophthalmologic screening for pediatric T1DM should begin at puberty when there is a possibility of developing diabetic retinopathy (8). In these guidelines, as the purpose of ophthalmologic screening is to detect the early stage of diabetic retinopathy, there is no mention of screening for OA associated with WS. In the present patient, the optic nerve head pallor enabled the diagnosis of WS. This abnormality can be detected on fundus examination. As a fundus examination is a minimally invasive test, ophthalmologic screening for younger patients with T1BDM may be useful for early diagnosis of WS. As in the present case, we propose that patients with T1BDM who have preserved endogenous insulin secretion may be eligible for ophthalmologic screening to investigate for WS. In patients with WS, the decline in endogenous insulin secretion is gradual and often maintained for a long time after diagnosis (9), whereas insulin secretion is usually depleted within a few years in patients with T1DM, especially in early childhood-onset cases (10).

Conclusion

Ophthalmologic screening can help in the early diagnosis of WS in patients with T1BDM and can facilitate effective treatment. The usefulness of this strategy for diagnosing WS should be validated in the future.

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Ethics

Informed Consent: Consent form was filled out by the parents.

Authorship Contributions

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References

1. Barrett TG, Bundey SE, Macleod AF. Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. Lancet 1995;346:1458-1463.
2. Li M, Wang S, Xu K, Chen Y, Fu Q, Gu Y, Shi Y, Zhang M, Sun M, Chen H, Han X, Li Y, Tang Z, Cai L, Li Z, Shi Y, Yang T, Polychronakos C. High Prevalence of a Monogenic Cause in Han Chinese Diagnosed With Type 1 Diabetes, Partly Driven by Nonsyndromic Recessive WFS1 Mutations. Diabetes 2020;69:121-126. Epub 2019 Oct 28
3. Marchand L, Li M, Leblicq C, Rafique I, Alarcon-Martinez T, Lange C, Rendon L, Tam E, Courville-Le Bouyonnec A, Polychronakos C. Monogenic Causes in the Type 1 Diabetes Genetics Consortium Cohort: Low Genetic Risk for Autoimmunity in Case Selection. J Clin Endocrinol Metab 2021;106:1804-1810.
4. Lin Y, Sheng H, Ting TH, Xu A, Yin X, Cheng J, Mei H, Shao Y, Zeng C, Zhang W, Rao M, Liu L, Li X. Molecular and clinical characteristics of monogenic diabetes mellitus in southern Chinese children with onset before 3 years of age. BMJ Open Diabetes Res Care 2020;8:001345.
5. Matsunaga K, Tanabe K, Inoue H, Okuya S, Ohta Y, Akiyama M, Taguchi A, Kora Y, Okayama N, Yamada Y, Wada Y, Amemiya S, Sugihara S, Nakao Y, Oka Y, Tanizawa Y. Wolfram syndrome in the Japanese population; molecular analysis of WFS1 gene and characterization of clinical features. PLoS One 2014;9:106906.
6. Zmyslowska A, Borowiec M, Fichna P, Iwaniszewska B, Majkowska L, Pietrzak I, Szalecki M, Szypowska A, Mlynarski W. Delayed recognition of Wolfram syndrome frequently misdiagnosed as type 1 diabetes with early chronic complications. Exp Clin Endocrinol Diabetes 2014;122:35-38. Epub 2014 Jan 24
7. Frontino G, Raouf T, Canarutto D, Tirelli E, Di Tonno R, Rigamonti A, Cascavilla ML, Baldoli C, Scotti R, Leocani L, Huang SC, Meschi F, Barera G, Broccoli V, Rossi G, Torchio S, Chimienti R, Bonfanti R, Piemonti L. Case Report: Off-Label Liraglutide Use in Children With Wolfram Syndrome Type 1: Extensive Characterization of Four Patients. Front Pediatr 2021;9:755365.
8. Donaghue KC, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, Zabeen B, Salem MA, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes 2018;19(Suppl 27):262-274.
9. Ray MK, Chen L, White NH, Ni R, Hershey T, Marshall BA. Longitudinal progression of diabetes mellitus in Wolfram syndrome: The Washington University Wolfram Research Clinic experience. Pediatr Diabetes 2022;23:212-218. Epub 2021 Dec 19
10. Barker A, Lauria A, Schloot N, Hosszufalusi N, Ludvigsson J, Mathieu C, Mauricio D, Nordwall M, Van der Schueren B, Mandrup-Poulsen T, Scherbaum WA, Weets I, Gorus FK, Wareham N, Leslie RD, Pozzilli P. Age-dependent decline of β-cell function in type 1 diabetes after diagnosis: a multi-centre longitudinal study. Diabetes Obes Metab 2014;16:262-267. Epub 2013 Oct 29

CASE REPORT

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Long-term Survival in a Child with Malignant Insulinoma After Liver Transplantation

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What is already known on this topic?

Insulinoma, especially malignant insulinoma, is a very rare, pancreatic neuroendocrine tumor in children. Insulinoma may be a part of multiple endocrine neoplasia type 1 but is very rare in von Hippel-Lindau syndrome, neurofibromatosis type 1, or tuberous sclerosis. Surgical resection remains the treatment of choice whenever possible. Diazoxide or somatostatin analogs (SSAs) can be used, either as an initial pre-surgical treatment or to achieve biochemical control in patients with unresectable tumors.

What this study adds?

Long-term survival and even remission may be achieved in malignant insulinoma metastatic to the liver in young patients if treated appropriately in experienced centers. It may require repeated surgical procedures, close monitoring and modification of anti-rejection therapies together with continued treatment with SSAs.

Abstract

Insulinoma is one of the pancreatic neuroendocrine tumors (PanNET) and is exceptionally rare in children. The tumor leads to severe hypoglycemia caused by excessive insulin release. We report a pediatric patient with malignant insulinoma who underwent liver transplantation (LT) due to liver metastases of the insulinoma. A 13-year-old girl presented with symptoms of hypoglycemia due to hyperinsulinism. On computed tomography (CT), a polycystic lesion in the head of the pancreas and enlarged lymph nodes were revealed. A modified Whipple's operation was performed, and histological examination confirmed PanNET. CT also showed an enlarged liver with numerous metastases. Allogeneic LT was carried out successfully. Positron emission tomography-CT using ^{68}Ga-DOTA-labeled somatostatin analogs (SSAs) at the age of 22 years confirmed complete metabolic remission. The patient currently remains under immunosuppressive and anti-proliferative treatment. Multiple surgical interventions, LT combined with SSAs, and immunosuppressive medication proved effective in this case of metastatic malignant insulinoma.

Keywords: Hypoglycemia, insulinoma, liver transplantation, children

Introduction

Insulinoma is an isolated, usually benign, pancreatic neuroendocrine tumor (PanNET) with an extremely low prevalence (annual incidence of 4 in every 1 million persons). Although this pancreatic islet mass may exhibit a range of symptoms, one of its main characteristics is the Whipple's triad, which consists of fasting hypoglycemia (< 50 mg/dL), symptoms of hypoglycaemia, and the disappearance of these symptoms after food intake. This condition may be associated with multiple endocrine neoplasia type 1

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(MEN-1) and, very rarely, with von Hippel-Lindau syndrome, neurofibromatosis type 1, or tuberous sclerosis.

Once the tumor is found through the diagnostic process, the leading medical procedure to be applied is surgical removal, which is used in over 90% of all recorded cases (1). However, it should be noted that the conventional examination can result in misdiagnosis, and the islet mass may not be detected at all. Abdominal ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) can not localize these frequently small tumors. If these imaging techniques do not visualize the lesion other methods include positron emission tomography (PET)/CT or PET/MRI (2).

Case Report

A 13-year old girl with no prior unusual medical history was admitted to the District Hospital with weakness, paleness, profuse sweating, balance impairment, and hypoglycaemia (35-43 mg/dL). Loss of consciousness and seizures were not observed. An intravenous glucose infusion was applied. Abdominal MRI revealed a polycystic heterogeneous mass with visible cystic areas just below the lobus caudatus of the liver and in the immediate vicinity of the pancreatic uncinate process.

The patient was transferred to the endocrinology department where no abnormalities were detected on initial examination. However, hypoglycaemia (27 mg/dL) was identified, without urinary acetone and with a correct hyperglycaemic response after the glucagon test with the marked level of insulin and C-peptide during hypoglycemia of 90.9 μIU/mL and 3.86 ng/mL, respectively (Table 1). The results indicated endogenous hyperinsulinism.

Subsequently, an intravenous infusion of 10% glucose and oral diazoxide (daily dose 5 mg/kg, divided into three equal doses every 8 hours) was administered. Due to side effects, including weight gain, recurrent headaches, and hirsutism, diazoxide treatment was stopped. Considering the possibility of MEN-1, prolactin, insulin-like growth factor-1, calcium, and parathyroid hormone were measured, and these results were within normal ranges. Furthermore, the genetic test for MEN-1 syndrome did not support the diagnosis. PET/CT showed only one mass with high metabolism of fluorine-18-L-3,4-dihydroxyphenylalanine (^{18}F-DOPA) in the head of the pancreas (Figure 1.1D), whereas the CT scan revealed a polycystic lesion in the head of the pancreas and metastases to the liver and lymph nodes (Figure 1.1A). The patient underwent a modified Whipple's operation based on the clinical picture and test results. Histopathological examination of the sample material collected during the procedure showed PanNET G2 with Ki-67 labeling index 16% (Figure 1.4A) and mitotic count 10 mitoses/mm² (Figure 1.3C), and with metastases to the liver and three peripancreatic lymph nodes. Immunohistochemical examination revealed the expression of cytokeratin (Figure 1.5A), chromogranin (Figure 1.5B), and synaptophysin in neoplastic cells. In addition, the tumor cells were positive for somatostatin (Figure 1.5C) and focally for insulin (Figure 1.5D).

Post-operational hypoglycemia occurred and was successfully treated with somatostatin analogs (SSAs) (30 mg of octreotide LAR, intramuscular injection every four weeks). Two months after the operation, a CT of the body was performed. It showed an enlarged liver with numerous metastases (Figure 1.1B, 1C). PET/CT using 68-gallium 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (^{68}Ga-DOTA)-labeled SSAs imaging revealed nine focal points of pathological intensification of the isotopic marker in both liver lobes. As a result, allogeneic liver transplantation (LT) was carried out, and sirolimus was given. PanNET G2 was then identified on histopathological examination of the liver and metastatic lymph nodes. Thirteen months after the graft, immunosuppressive treatment was modified to tacrolimus due to cytomegalovirus infection. Four years after diagnosis, ^{68}Ga-DOTA PET/CT revealed two metastatic foci (Figure 1.2A-2D).

Table 1. Blood samples results at first presentation

Parameters	Serum level	Reference range
Blood glucose (mg/dL)	27	70-99
Insulin (μIU/mL)	90.9	4-16
C-peptide (ng/mL)	3.86	0.5-2.0
β-hydroxybutyrate (mg/dL)	0.43	0.21-2.81
Lactates (mmol/L)	2.34	0.5-1.6
Glucagon test-blood glucose (mg/dL)	0'-35 5'-40 15'-61 30'-100	An increase in glucose of >25 mg/dL suggests an insulin-mediated etiology
Morning cortisol (μg/dL)	33.74	5-20
Prolactin (ng/mL)	23.10	5.18-26.53
IGF-1 (ng/mL)	384.0	183-850
Calcitonin (ng/L)	<0.9	0.5-7.8
Calcium (mmol/L)	2.41	2.10-2.55
Phosphorus (mmol/L)	1.22	0.95-1.75
PTH (pg/mL)	82.0	16-87
αFP (IU/mL)	2.61	<5
β-HCG (mIU/mL)	<0.03	<0.1
CgA (U/l)	9	2-18
NSE (μg/L)	13.7	<18.3

IGF-1: insulin like growth factor-1, PTH: parathyroid hormone, αFP: α-fetoprotein, β-HCG: β-human chorionic gonadotropin, CgA: chromogranin A, NSE: neuron-specific enolase

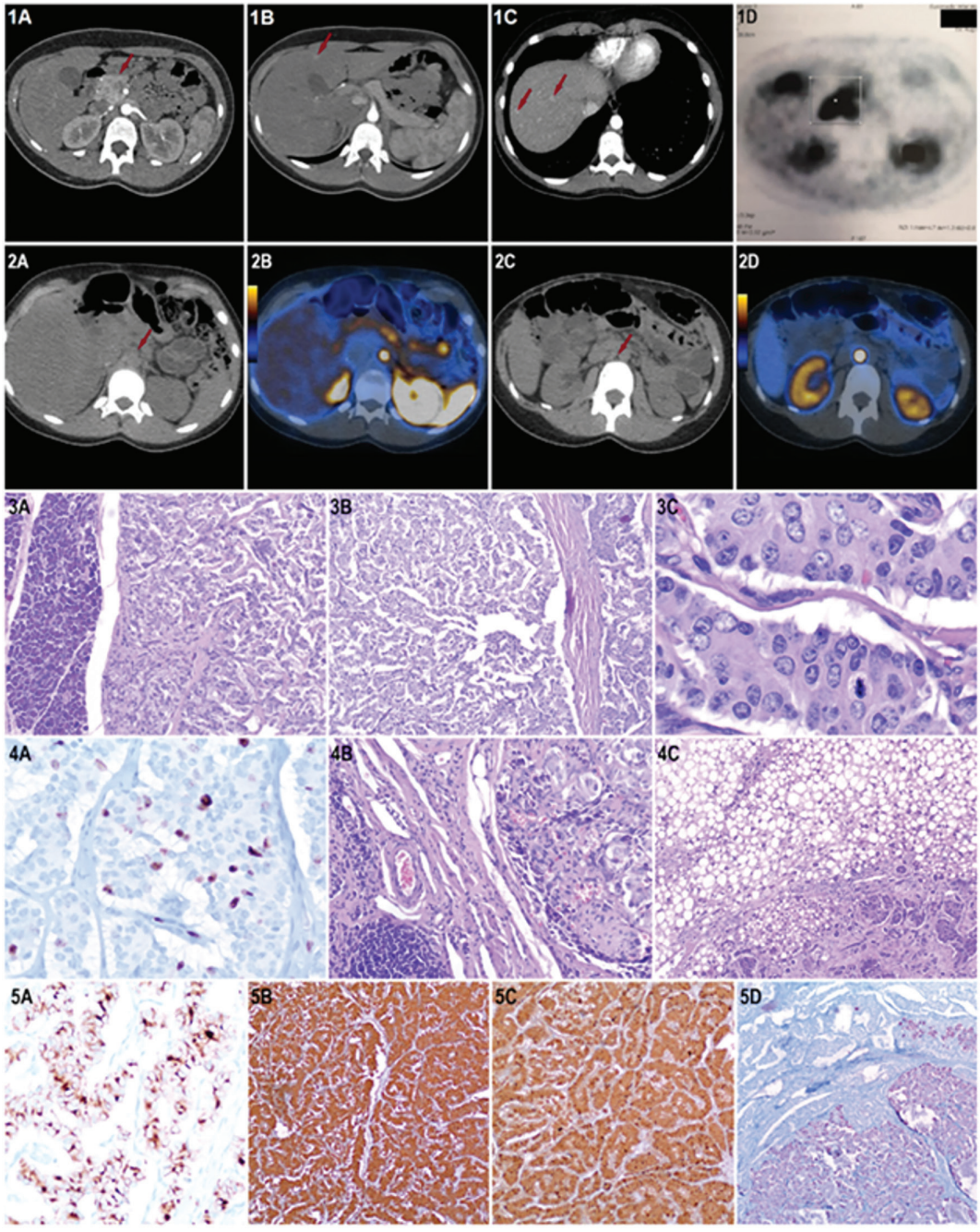

Figure 1. 1A-D: Imaging tests (CT, PET/CT) before (1A, 1D) and 2 months after (1B, 1C) excision of pancreatic neuroendocrine tumor. 1A: CT-a polycyclic lesion 27x36x43 mm in the head of the pancreas, widening of the pancreatic duct and enlarged lymph nodes in the hilum area of the liver (up to 14 mm) (red arrow). 1B-C: CT-metastases to the liver of the diameter reaching 2.5 cm and numerous lymph nodes (red arrow) in the area of adipose tissue of mesenterium. 1D: PET/CT-the intensified uptake focus of ^{18}F-DOPA in the head of the pancreas. 2A-D: ^{68}Ga-DOTA PET/CT-two foci of increased somatostatin analogue uptake identified after 4 years from diagnosis. 2A-B: ^{68}Ga-DOTA PET/CT-from the left side of the superior mesenteric artery (red arrow). 2C, 2D: ^{68}Ga-DOTA PET/CT-between aorta and inferior vena cava on the level of L2 (red arrow). 3,4A-C: Hematoxylin and eosin staining of tumor cells of pancreatic material collected during the first surgical procedure. 3A. The border region between tumor and pancreas. 3B: Trabecular architecture. 3C: Mitotic activity of neoplastic cells (10 mitoses/2 mm^2). 4A: Ki67 labeling index of neoplastic cells (16%). 4B: The regional lymph node with metastases. 4C: The liver with steatosis and the presence of metastases. 5A-D: Immunochemical examination of tumor cells of pancreatic material collected during the first surgical procedure. 5A: Tumor cells stained positive for cytokeratin. 5B: Tumor cells stained positive for chromogranin. 5C: Tumor cells stained positive for somatostatin. 5D: Tumor cells stained positive for insulin

PET/CT: positron emission tomography/computed tomography

The patient had a laparotomy to remove these metastases, including the the paraaortic lymph node bundle and the small lymph node between the left adrenal gland, renal artery, and aorta. Histopathological examination confirmed metastatic foci of neuroendocrine tumor in the paraaortic lymph nodes (NET G2).

The result of ^{68}Ga-DOTA-labeled SSAs PET/CT is currently, at the age of 22 years, negative for active neoplastic disease with an increased expression level of somatostatin receptors, which indicates complete metabolic remission is maintained. At present, after three operations, the patient has been in remission for over six years, taking immunosuppressive medication (mycophenolate mofetil) due to liver transplant and anti-proliferative treatment (SSA and rapamycin).

Written informed consent was obtained from the patient.

Discussion

Insulinoma is an uncommon neuroendocrine tumor of the pancreas, characterized by autonomous insulin secretion by islet beta cells regardless of glycaemic status. At diagnosis, the median age is 47 years (2) but it may occur in any age group. Insulinomas are usually solitary, sporadic benign tumors, and less than 10% are malignant (3,4). Moreover, about 10% of insulinomas are associated with MEN-1 (2).

The most characteristic finding is fasting hypoglycemia, often after exercise or prolonged fasting. Sympathoadrenal activation symptoms may be evident, including palpitations, tremors, and sweating. Severe hypoglycemia can cause neuroglycopenic symptoms, such as blurry vision, cognitive impairment, or seizures.

The suspicion of insulinoma is based on Whipple's triad and inappropriately elevated blood insulin levels with hypoglycemia during the fasting test. Establishing a diagnosis of an insulinoma requires demonstrating inappropriately high insulin, proinsulin, or C-peptide levels during hypoglycemia in a fasting test. Furthermore, after intravenous glucagon administration, beta-hydroxybutyrate levels below 2.7 mmol/L and glycemia above 25 mg/dL (1.4 mmol/L) help establish the diagnosis (5).

Malignant insulinoma is extremely rare in a pediatric population, and metastases are mainly observed in the liver and regional lymph nodes (6). In the differential diagnosis of hypoglycemia in children, the presence of acidemia is essential. If non-ketotic hypoglycemia is suspected, defects of ketogenesis, such as carnitine deficiency or beta-oxidation defects, like medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD) presenting with high free fatty acid and low insulin levels, should be considered (7). Given that congenital hyperinsulinism usually manifests in the neonatal period and that hypoglycemia occurred at the age of 13 years in our patient, this diagnosis was very unlikely. Moreover, hypoglycemia may be a manifestation of pituitary or adrenal deficiency and may also be observed during sulfonylurea or insulin treatment (5).

Localization of insulinoma is challenging and requires both invasive and non-invasive imaging, though the sensitivities and specificities are not well documented in children. The most commonly used techniques include 3-phase CT, MRI, and endoscopic ultrasound (5). The effectiveness of CT and MRI in detecting insulinoma in adults is estimated at 55% and 61% respectively (8).

Other diagnostic options include somatostatin receptor scintigraphy, although tumors often lack sufficient expression of somatostatin receptors, especially somatostatin receptor subtype 2. However, certain insulinomas express the glucagon-like peptide-1 receptor. If the imaging techniques mentioned above do not visualize the lesion, other methods, including PET/CT or PET/MRI using ^{68}Ga-DOTA-labeled SSAs, ^{18}F-DOPA PET/CT, or ^{68}Ga-DOTA-exendin-4 PET/CT may be employed (5). These methods significantly improved the effectiveness of localizing insulinoma, reported by some authors to be >90% (8,9). Invasive regionalization procedures, including arterial stimulation and venous sampling or trans-hepatic portal venous sampling, are currently less used because of the continuous development of imaging techniques (5).

Surgical intervention is the treatment of choice, which permits a cure. Insulinomas are typically removed by enucleation of the tumor. Rarely, tumors located in the head of the pancreas require a pancreaticoduodenectomy (Whipple's procedure), as in the presented patient. Moreover, numerous liver metastases prompted the decision to perform LT in the present case, based on Milan's criteria (10). To date, several malignant, metastatic insulinoma cases in children, which required LT, have been published (11,12).

Medical management of insulinoma consists of the initial pre-surgical treatment or achieving biochemical control in patients with unresectable tumors. Diazoxide, which inhibits insulin secretion and enhances glycogenolysis, may be the first-line treatment. Side effects, such as sodium retention, edema, congestive heart failure, or hirsutism, are observed, though these are usually not severe. The addition of diuretic benzothiadiazine, which improves the hyperglycaemic effect of diazoxide and reduces edema, should be considered. Glycaemic control can be achieved with calcium channel blockers, beta-adrenergic-receptor

blocking drugs or glucocorticoids in selected patients (5). SSAs are an important part of insulinoma therapy due to their inhibition of secretion of insulin and anti-proliferating effects. SSAs slow down the progression of the disease and reduce the size of the tumor (5). Despite incomplete resection after numerous surgical procedures, SSAs maintained remission in our patient.

For advanced, unresectable cases, other types of therapy, such as peptide receptor radionuclide therapy, tyrosine kinase inhibitors, and inhibitors of the mammalian target of rapamycin, or chemotherapy can still be used, especially in adults (5).

Conclusion

In conclusion, malignant insulinoma is a rare tumor in children. Surgical intervention remains the treatment of choice whenever possible. In case of incomplete resection or recurrence, multiple surgical interventions, pharmacological treatment, or chemotherapy should be considered. The risk of recurrence makes long-term follow-up mandatory. However, even with advanced, metastatic disease, current treatment options may facilitate maintenance of complete remission for many years, as in the presented patient.

Ethics

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

Authorship Contributions

Surgical and Medical Practices: Elżbieta Moszczyńska, Arnika Wydra, Klaudia Zasada, Marta Baszyńska-Wilk, Dorota Majak, Anna Śliwińska, Wiesława Grajkowska, Concept: Elżbieta Moszczyńska, Arnika Wydra, Design: Elżbieta Moszczyńska, Arnika Wydra, Data Collection or Processing: Elżbieta Moszczyńska, Arnika Wydra, Klaudia Zasada, Marta Baszyńska-Wilk, Dorota Majak, Anna Śliwińska, Wiesława Grajkowska, Analysis or interpretation: Elżbieta Moszyczńska, Arnika Wydra, Literature Search: Elżbieta Moszczyńska, Arnika Wydra, Writing: Elżbieta Moszcyńska, Arnika Wydra.

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References

1. Sun M, Luo Y, You Y, Han X, Zhao Y, Han X, Zhao Y. Ectopic insulinoma: case report. BMC Sur 2019;19:197.
2. Service FJ, McMahon MM, O'Brien PC, Ballard DJ. Functioning insulinoma--incidence, recurrence, and long-term survival of patients: a 60-year study. Mayo Clin Proc 1991;66:711-719.
3. Janem W, Sultan I, Ajlouni F, Deebajeh R, Haddad H, Sughayer MA, Goussous RY. Malignant insulinoma in a child. Pediatr Blood Cancer 2010;55:1423-1426.
4. Jung SY, Kang B, Choi YM, Kim JM, Kim SK, Kwon YS, Lee JE. Development of multifocal nodular lesions of a liver mimicking hepatic metastasis, following resection of an insulinoma in a child. Korean J Pediatr 2015;58:69-72. Epub 2015 Feb 28
5. Herder WW de, Zandee WT, Hofland J. Insulinoma [Internet]. Endotext [Internet]. MDText.com, Inc.; 2020 [cited 2022 Jan 9]. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK278981/
6. Okabayashi T, Shima Y, Sumiyoshi T, Kozuki A, Ito S, Ogawa Y, Kobayashi M, Hanazaki K. Diagnosis and management of insulinoma. World J Gastroenterol 2013;19:829-837.
7. Gozzi Graf T, Brändle M, Clerici T, l'Allemand D. Insulinoma: only in adults?-case reports and literature review. Eur J Pediatr 2014;173:567-574. Epub 2013 Apr 21
8. Nockel P, Babic B, Millo C, Herscovitch P, Patel D, Nilubol N, Sadowski SM, Cochran C, Gorden P, Kebebew E. Localization of Insulinoma Using 68Ga-DOTATATE PET/CT Scan. J Clin Endocrinol Metab 2017;102:195-199.
9. Christ E, Antwi K, Fani M, Wild D. Innovative imaging of insulinoma: the end of sampling? A review. Endocr Relat Cancer 2020;27:79-92.
10. Sposito C, Droz Dit Busset M, Citterio D, Bongini M, Mazzaferro V. The place of liver transplantation in the treatment of hepatic metastases from neuroendocrine tumors: Pros and cons. Rev Endocr Metab Disord 2017;18:473-483.
11. Lobe TE, Vera SR, Bowman LC, Fontanesi J, Britt LG, Gaber AO. Hepaticopancreaticogastroduodenectomy with transplantation for metastatic islet cell carcinoma in childhood. J Pediatr Surg 1992;27:227-229.
12. Vossen S, Goretzki PE, Goebel U, Willnow U. Therapeutic management of rare malignant pancreatic tumors in children. World J Surg 1998;22:879-882.

CASE REPORT

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Gastroparesis in Adolescent Patient with Type 1 Diabetes: Severe Presentation of a Rare Pediatric Complication

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What is already known on this topic?

Gastroparesis is a long-term complication of poorly controlled diabetes, which can also evolve into potentially life-threatening conditions, including dehydration, malnutrition, and electrolyte imbalance.

What this study adds?

Although gastroparesis is more frequent in adults, it may also affect pediatric patients with type 1 diabetes. The use of technology, especially of hybrid closed-loop systems, can be helpful in the management of these patients, as in the presented case.

Abstract

Gastroparesis is a long-term complication of diabetes related to autonomic neuropathy. It is characterized clinically by delayed gastric emptying and upper gastrointestinal symptoms, including early satiety, postprandial fullness, nausea, vomiting, and abdominal pain. Gastric emptying scintigraphy is the gold standard for diagnosis as it reveals delayed gastric emptying. Therapeutic strategies include dietary modifications, improvement of glycemic control, and prokinetic drugs. Case descriptions of diabetic gastroparesis in pediatric ages are very scarce. We report the case of a 16-year-old adolescent with severe presentation of diabetic gastroparesis. She presented with recurrent episodes of nausea, vomiting and abdominal pain which led progressively to reduced oral intake and weight loss. Her past glycemic control had been quite brittle, as demonstrated by several hospitalizations due to diabetic ketoacidosis and recurrent episodes of severe hypoglycemia. After the exclusion of infectious, mechanical, metabolic, and neurological causes of vomiting, a gastric emptying scintigraphy was performed, leading to the diagnosis of gastroparesis. Treatment with metoclopramide was started with progressive relief of symptoms. To improve glycemic control, insulin therapy with an advanced hybrid, closed loop system was successfully started. Pediatricians should consider diabetic gastroparesis in children and adolescents with long-standing, poorly controlled diabetes and appropriate symptomology.

Keywords: Advanced hybrid closed-loop, gastric emptying, metoclopramide, microvascular complications, scintigraphy

Introduction

Long-term complications of type 1 diabetes (T1D) include retinopathy, nephropathy, neuropathy, and macrovascular disease. Clinical manifestations of these complications are rarely observed in the pediatric population, but early alterations can be detected even in the first years of disease, among children and adolescents with poor glycemic control. Therefore, screening strategies and therapeutic optimization are fundamental in young patients to prevent progression towards advanced stages of complications (1).

Diabetic neuropathy can affect both somatic and autonomic nerves. The most common form is diabetic sensorimotor polyneuropathy, characterized by progressive damage of

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peripheral nerves, usually starting with sensory fibers and thereafter involving motor fibers (2). The first symptoms usually include numbness, prickling, paresthesia, and burning of upper and lower limbs. According to the latest ISPAD guidelines (1), screening of peripheral neuropathy should start at the age of 11 years in patients with 2 to 5 years of diabetes duration, and then should be performed every year. Screening should include a careful clinical neurological evaluation and electrophysiological tests. Therapy for neuropathy is often personalized and modified according to the effectiveness and safety for each patient. In the early stages, the most commonly used drugs to relieve diabetic neuropathic pain are pregabalin, duloxetine, and gabapentin (2).

Autonomic neuropathy can affect many nerves, including those innervating cardiovascular, urinary, and gastrointestinal systems (3). Cardiovascular autonomic neuropathy is the most prevalent form of autonomic impairment, affecting at least 20% of people with diabetes of all ages (4). Orthostatic hypotension and abnormal heart rate response are the most common manifestations of this complication, which is related to a high risk of death due to life-threatening arrhythmias and sudden cardiac death (5). Moreover, an impaired mechanism of sympathetic response to hypoglycemia has been suggested in patients with autonomic neuropathy, which may lead to the onset of unawareness of hypoglycemia (6,7). Finally, autonomic neuropathy may also result in gastrointestinal involvement, known as diabetic gastroparesis. An impaired interaction between the enteric nervous system and gut-brain axis causes the occurrence of this insidious complication (8).

Case Report

We present the case of a 16-year-old girl with T1D, diagnosed when she was two years old. Since the onset of diabetes she has been on multiple daily injection therapy and followed at a local pediatric diabetes service. Glycemic control has been quite brittle, as demonstrated by several hospitalizations due to diabetic ketoacidosis and recurrent episodes of severe hypoglycemia. Screening tests for common chronic diabetic complications had been never performed. The patient was admitted at our department due to a three year history of recurrent episodes of nausea, vomiting, and abdominal pain. Family history was negative for gastrointestinal and autoimmune diseases. Gastrointestinal symptoms were mainly associated to solid meals and had drastically worsened in the preceding months, leading to reduced oral intake and weight loss. Vomiting episodes had no stereotypical characteristics, predictable timing or associated neurovegetative symptoms. She also had constipation that was unresponsive to polyethylene glycol treatment.

At admission, she suffered from abdominal pain, which was exacerbated by palpation. On clinical examination, she had mild dehydration. No other relevant findings were present. At presentation she weighed 53.6 kg [-0.25 standard deviation score (SDS)], had a height of 160.2 cm (-0.50 SDS), and her body mass index was 20.9 kg/m^2 (+0.17 SDS). Her pubertal development was complete. Complete blood count and blood biochemistry analyses, including liver function, renal function, pancreatic enzymes, electrolytes, blood ketones, inflammatory indices, thyroid hormones, plasma ammonia concentration, toxicological tests, and β-human chorionic gonadatropin were normal or negative. Glycated hemoglobin (HbA1c) was 9.9% (85 mmol/mol). Screening for celiac disease was negative. Normal levels of adrenocorticotropic hormone, serum cortisol, and plasma renin ruled out adrenal insufficiency. Neurological causes of vomiting were excluded by performing brain magnetic resonance imaging and electroencephalogram. Plain abdominal radiography showed stool burden without any sign of intestinal obstruction or perforation. A computed tomography scan with contrast excluded extrinsic causes of gastric outlet obstruction, including superior mesenteric artery syndrome. An upper endoscopy was performed, revealing moderate, non-specific gastritis. No obstruction in the upper gastrointestinal tract was present and no esophageal or duodenal lesion was evident. Autoimmune gastritis was excluded because of normal levels of vitamin B12 and negative anti-gastric parietal cell antibodies. Treatment with proton pump inhibitor and ondansetron at the maximum dosage of 30 mg/day was started without any benefits. Oral feeding was replaced by total parenteral nutrition. To minimize the blood glucose instability continuous intravenous administration of regular insulin was started.

A gastric emptying scintigraphy after administration of a liquid meal radiolabeled with technetium-99mTc-diethylenetriaminepentaacetic acid was then performed, revealing gastric meal retention (Figure 1). On the basis of her history, characterized by prolonged poor glycemic control, and laboratory and radiographic findings, a diagnosis of diabetic gastroparesis was definitively made and treatment with metoclopramide, starting at the dose of 30 mg/day was begun.

In the following days, a progressive improvement of symptoms was recorded, and liquid and solid foods were reintroduced gradually, being well tolerated by the patient. Oral polyethylene glycol was then administered, with progressive regularization of bowel movements. To reduce

glycemic excursions, insulin therapy with an advanced hybrid closed loop (aHCL) system (Medtronic MiniMed 780G™; Medtronic Diabetes, Northridge, CA, USA) was started, achieving a prompt improvement of glycemic control, as demonstrated in Figure 2.

To evaluate the presence of other signs of early diabetic complications, nerve conduction studies were performed, revealing the presence of peripheral neuropathy affecting mainly lower limbs. Supplementation with B complex vitamins, folate and uridine was then started. An early

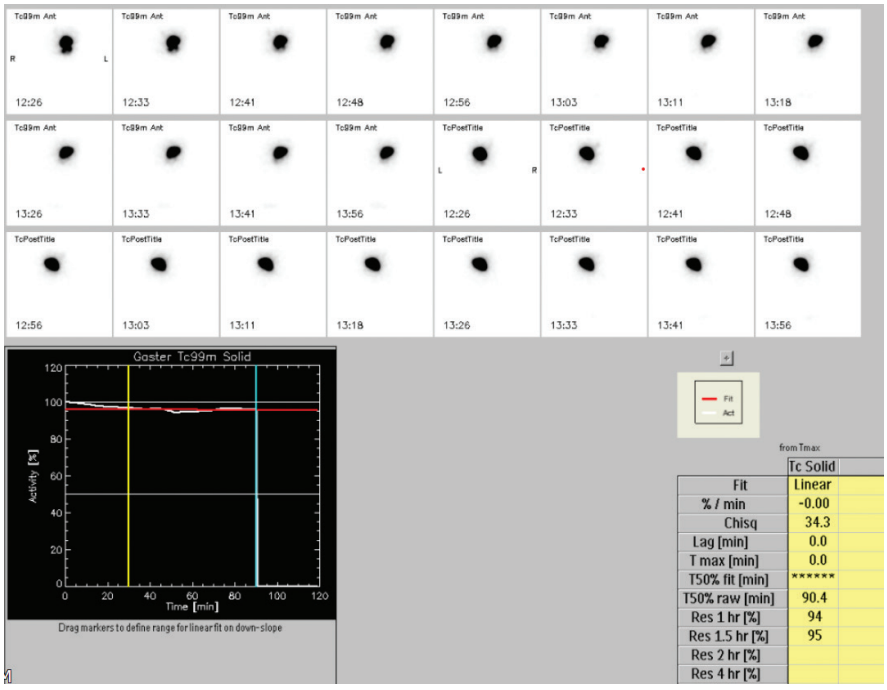

Figure 1. Upper panel: anterior and posterior planar images of the gastric contents up to 90 minutes after ingestion of the liquid bolus. Lower panel: the activity/time curve of the gastric contents shows no significant deflection

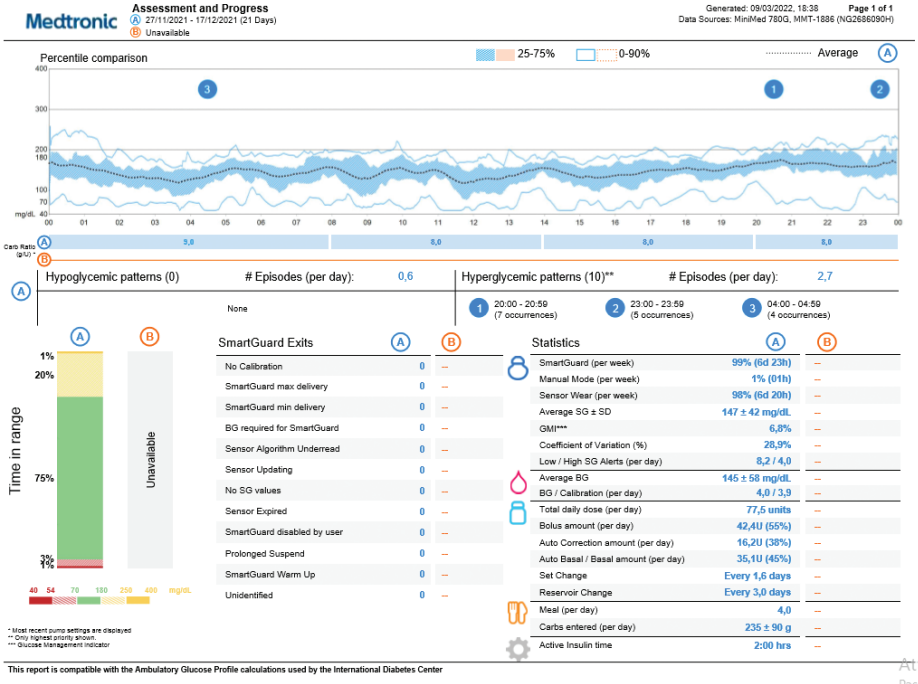

Figure 2. Assessment of glucose control during the first three weeks of advanced hybrid closed loop system use. All glucose metrics met the recommended clinical targets. Data were extracted from CareLink™ system software

stage retinopathy was also evident on fundus examination. Diabetic nephropathy was ruled out by the normality of albuminuria in a 24-hour urine collection.

The dose of metoclopramide was gradually reduced until complete withdrawal after three weeks of treatment. Therapy on demand with domperidone was then recommended in the case of relapse of symptoms.

At the three-month follow-up visit after discharge, the patient showed persistent remission of gastrointestinal symptoms, a weight gain of 5.9 kg, and improved quality of life. She is currently being followed up at an ophthalmological center for further investigations relating to her retinopathy.

Discussion

Gastroparesis is a clinical condition characterized by delayed gastric emptying and upper gastrointestinal symptoms, including early satiety, postprandial fullness, nausea, vomiting, and abdominal pain. Clinical manifestations are heterogeneous, ranging from mild symptoms to potentially life-threatening conditions such as dehydration, malnutrition, and electrolyte imbalance (9).

Pathogenesis of diabetic gastroparesis involves autonomic neuropathy and enteric neuromuscular system damage caused by different mechanisms including oxidative stress, hyperglycemia, and inflammation (8). An independent negative effect of acute hyperglycemia on gastric emptying time has been also hypothesized (10).

Diabetic gastroparesis may adversely affect the management of diabetes. Its pathogenetic mechanism is associated to glycemic control through a bidirectional causal relationship: long-term poor glycemic control and acute hyperglycemia can both cause gastric emptying delay and gastroparesis, and *vice versa* gastrointestinal dysfunction can affect glycemic variability of patients (10,11). Indeed, published data show that patients with diabetic gastroparesis present an increased risk of hypoglycemia (12), especially in the post-prandial period. This phenomenon has been named "gastric hypoglycemia" and seems to be related to a mismatch between prandial insulin absorption and postprandial increase of blood glucose, which is delayed in patients with gastroparesis (13).

There are few published data about prevalence of diabetic gastroparesis, and none of them consider the pediatric population. Within the largest adult T1D clinical registry of the United States, 4.8% of patients had a clinical diagnosis of gastroparesis (14). A community based study revealed a cumulative incidence of diabetic gastroparesis during 10 years of 5% in patients with T1D, and a greater risk in comparison to type 2 diabetes (15). Case descriptions of diabetic gastroparesis in pediatric patients are very scarce (16). To the best of our knowledge, this is the first report with such a severe presentation in an adolescent.

Gastric emptying scintigraphy is currently considered the gold standard for the diagnosis of gastroparesis in both children and adults (17,18). Gastric scintigraphy is considered diagnostic when it detects a retention of >90% of the radiolabeled meal after the first hour of exam (19). Mechanical gastric outlet obstruction should be ruled out prior to the scintigraphy by performing an upper endoscopy (20).

Therapeutic strategies for diabetic gastroparesis include dietary modifications, prokinetic drugs, and improvement of glycemic control. Modifications of diet are mandatory to facilitate gastric emptying and generally consist of the consumption of small meals and the avoidance of fibers and high-fat foods (21). Pharmacological therapy with prokinetic drugs is fundamental in the management of these patients. Dopamine agonists, including metoclopramide and domperidone, are widely used for the treatment of diabetic gastroparesis in adult patients, as they have been shown to relieve symptoms of nausea and vomiting and to accelerate gastric emptying (22,23). However, the use of metoclopramide in pediatric patients should be carefully evaluated and is not recommended for the long-term, due to the high risk of developing severe extrapyramidal side effects (24).

Optimization of glycemic control has been also demonstrated to improve symptoms of gastroparesis (10). Moreover, lower levels of HbA1c have been shown to be associated with reduced gastrointestinal symptoms and with faster gastric emptying (25). In the presented case, we decided to start continuous subcutaneous insulin infusion therapy with an aHCL system. Two studies have already demonstrated the efficacy of HCL systems in adults with T1D and gastroparesis (26,27). In particular, Daly et al. (26) recently reported significant reductions in HbA1c and mean glucose in five patients over one year of HCL use, while Kaur et al. (27) had previously shown that HCL had similar effectiveness and safety in adults with T1D and gastroparesis compared to age, sex, and diabetes duration controls.

Conclusion

The present case suggests that diabetic gastroparesis, with its wide range of possible clinical presentations, should be kept in mind by pediatricians managing children and adolescents with long-standing, poorly controlled diabetes.

Finally, detection of delayed gastric emptying should alert clinicians to the possible coexistence of other microvascular complications.

Ethics

Informed Consent: Written informed consent was obtained from the patient's parents for the publication of both clinical information and imaging.

Authorship Contributions

Surgical and Medical Practices: Stefano Costa, Mariella Valenzise, Nino Giannitto, Davide Cardile, Sergio Baldari, Writing: Fortunato Lombardo, Bruno Bombaci, Giuseppina Salzano, Stefano Passanisi.

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References

1. Donaghue KC, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, Zabeen B, Salem MA, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes 2018;19(Suppl 27):262-274.
2. Piona C, Ventrici C, Marcovecchio L, Chiarelli F, Maffeis C, Bonfanti R, Rabbone I. Long-term complications of type 1 diabetes: what do we know and what do we need to understand? Minerva Pediatr (Torino) 2021;73:504-522. Epub 2021 Sep 16
3. Trotta D, Verrotti A, Salladini C, Chiarelli F. Diabetic neuropathy in children and adolescents. Pediatr Diabetes 2004;5:44-57.
4. Spallone V. Update on the Impact, Diagnosis and Management of Cardiovascular Autonomic Neuropathy in Diabetes: What Is Defined, What Is New, and What Is Unmet. Diabetes Metab J 2019;43:3-30.
5. Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. Diabetes Care 2003;26:1895-1901.
6. Ewing DJ, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. Q J Med 1980;49:95-108.
7. Hepburn DA, Patrick AW, Brash HM, Thomson I, Frier BM. Hypoglycaemia unawareness in type 1 diabetes: a lower plasma glucose is required to stimulate sympatho-adrenal activation. Diabet Med 1991;8:934-945.
8. Meling S, Bertoli D, Sangnes DA, Brock C, Drewes A, Ejskjaer N, Dimcevski G, Søfteland E. Diabetic Gastroenteropathy: Soothe the Symptoms or Unravel a Cure? Curr Diabetes Rev 2022;18:e220321192412.
9. Stevens JE, Jones KL, Rayner CK, Horowitz M. Pathophysiology and pharmacotherapy of gastroparesis: current and future perspectives. Expert Opin Pharmacother 2013;14:1171-1186. Epub 2013 May 11
10. Halland M, Bharucha AE. Relationship Between Control of Glycemia and Gastric Emptying Disturbances in Diabetes Mellitus. Clin Gastroenterol Hepatol 2016;14:929-936. Epub 2015 Dec 21
11. Bharucha AE, Kudva YC, Prichard DO. Diabetic Gastroparesis. Endocr Rev 2019;40:1318-1352.
12. Ishii M, Nakamura T, Kasai F, Onuma T, Baba T, Takebe K. Altered postprandial insulin requirement in IDDM patients with gastroparesis. Diabetes Care 1994;17:901-903.
13. Horowitz M, Jones KL, Rayner CK, Read NW. 'Gastric' hypoglycaemia--an important concept in diabetes management. Neurogastroenterol Motil 2006;18:405-407.
14. Aleppo G, Calhoun P, Foster NC, Maahs DM, Shah VN, Miller KM; T1D Exchange Clinic Network. Reported gastroparesis in adults with type 1 diabetes (T1D) from the T1D Exchange clinic registry. J Diabetes Complications 2017;31:1669-1673. Epub 2017 Sep 6
15. Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Melton LJ 3rd, Talley NJ. Risk of gastroparesis in subjects with type 1 and 2 diabetes in the general population. Am J Gastroenterol 2012;107:82-88. Epub 2011 Nov 15
16. Reid B, DiLorenzo C, Travis L, Flores AF, Grill BB, Hyman PE. Diabetic gastroparesis due to postprandial antral hypomotility in childhood. Pediatrics 1992;90:43-46.
17. Abell TL, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ Jr, Ziessman HA; American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Am J Gastroenterol 2008;103:753-763. Epub 2007 Nov 19
18. Santhanam P, Marashdeh W, Solnes L. Functional Imaging of Evaluation of Diabetic Gastroparesis. Curr Diabetes Rev 2018;14:222-226.
19. Tougas G, Eaker EY, Abell TL, Abrahamsson H, Boivin M, Chen J, Hocking MP, Quigley EM, Koch KL, Tokayer AZ, Stanghellini V, Chen Y, Huizinga JD, Rydén J, Bourgeois I, McCallum RW. Assessment of gastric emptying using a low fat meal: establishment of international control values. Am J Gastroenterol 2000;95:1456-1462.
20. ASGE Standards of Practice Committee; Fukami N, Anderson MA, Khan K, Harrison ME, Appalaneni V, Ben-Menachem T, Decker GA, Fanelli RD, Fisher L, Ikenberry SO, Jain R, Jue TL, Krinsky ML, Maple JT, Sharaf RN, Dominitz JA. The role of endoscopy in gastroduodenal obstruction and gastroparesis. Gastrointest Endosc 2011;74:13-21.
21. Camilleri M. Functional Dyspepsia and Gastroparesis. Dig Dis 2016;34:491-499. Epub 2016 Jun 22
22. McCallum RW, Ricci DA, Rakatansky H, Behar J, Rhodes JB, Salen G, Deren J, Ippoliti A, Olsen HW, Falchuk K, et al. A multicenter placebo-controlled clinical trial of oral metoclopramide in diabetic gastroparesis. Diabetes Care 1983;6:463-467.
23. Heckert J, Parkman HP. Therapeutic response to domperidone in gastroparesis: A prospective study using the GCSI-daily diary. Neurogastroenterol Motil 2018:30. Epub 2017 Nov 7
24. Lau Moon Lin M, Robinson PD, Flank J, Sung L, Dupuis LL. The Safety of Metoclopramide in Children: A Systematic Review and Meta-Analysis. Drug Saf 2016;39:675-687.
25. Bytzer P, Talley NJ, Leemon M, Young LJ, Jones MP, Horowitz M. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. Arch Intern Med 2001;161:1989-1996.
26. Daly A, Hartnell S, Boughton CK, Evans M. Hybrid Closed-loop to Manage Gastroparesis in People With Type 1 Diabetes: a Case Series. J Diabetes Sci Technol 2021;15:1216-1223. Epub 2021 Aug 11
27. Kaur H, Schneider N, Pyle L, Campbell K, Akturk HK, Shah VN. Efficacy of Hybrid Closed-Loop System in Adults with Type 1 Diabetes and Gastroparesis. Diabetes Technol Ther 2019;21:736-739. Epub 2019 Aug 16

CASE REPORT

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Impact of Early Intervention with Triiodothyroacetic Acid on Peripheral and Neurodevelopmental Findings in a Boy with MCT8 Deficiency

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What is already known on this topic?

Thyroid function tests suggestive of central hypothyroidism in a boy with infantile hypotonia should alert the clinician to measure free triiodothyronine (FT3), while monocarboxylate transporter 8 (MCT8) deficiency should be in the differential diagnosis as early recognition and intervention is crucial. T3 and its analogue, Triac are structurally similar. Many commercial total T3 and FT3 assays are shown to cross-react significantly with Triac in a dose-dependent manner. Triac is known to improve clinical and biochemical signs of hyperthyroidism in patients with MCT8 deficiency by 12 months of treatment. However, earlier trials on efficacy of Triac were not designed to detect neurodevelopmental outcomes and age range of the study populations was diverse.

What this study adds?

After one year of Triac treatment, a significant change in neurodevelopmental scores were not recorded in the presented MCT8 deficient patient. However clinical improvement in critical developmental milestones were evident including at six months habituating to objects, recognizing and smiling at his mother, responding to a person's voice by turning his head, raising his face while in prone position and holding his head steady for a few seconds while in sitting position, and at 12 months responding to his name by turning his head, following a person across the room with eyes and head, and holding his head steady for at least 15 seconds. Regression was not observed. Early intervention with disease-modifying treatment, Triac, enables advances in peripheral findings of MCT8 deficiency as well as neurodevelopmental outcomes and may alleviate the risk factors of morbidity and mortality. Unexpectedly high FT3 in a patient receiving Triac should alert the clinician about a possible interference with FT3 when measured by immunoassay. If signs of hyperthyroidism are absent, patients should have their thyroid function test measured by mass spectrometry without a change in dose, keeping cross-reactivity in mind.

Abstract

Monocarboxylate transporter 8 (MCT8) deficiency is a rare genetic disorder characterized by peripheral thyrotoxicosis and severe cognitive and motor disability due to cerebral hypothyroidism. 3,3',5-triiodothyroacetic acid (Triac) was shown to improve peripheral thyrotoxicosis but data on neurodevelopmental outcome are scarce. We present a case of MCT8 deficiency and the experience with Triac focusing on change in neurodevelopmental and peripheral features. A five-month-old boy was referred because of feeding difficulty, central hypotonia and global developmental delay. Despite six months of physiotherapy, physical developmental milestones did not improve, and distal muscle tone was increased. A hemizygous pathogenic variant in *SLC16A2* was found and MCT8 deficiency was confirmed at 19-months. Thyroid stimulating hormone was 2.83 mIU/mL, free thyroxine 6.24 pmol/L (N = 12-22) and free triiodothyronine (FT3) 15.65pmol/L (N = 3.1-6.8). He had tachycardia, blood pressure and transaminases were elevated. Triac was started at 21-months. Two weeks after treatment, FT3 dramatically decreased, steady normal serum FT3 was achieved at 28-months. Assessment of neurodevelopmental milestones and signs of hyperthyroidism were evaluated at baseline, 6 months and 12 months after treatment. Signs of hyperthyroidism were improved by 6 months. Developmental composite scores of Bayley Scales of Infant Developmental 3rd Edition remained the same

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but important developmental milestones (head control, recognition of caregiver, response to his name) were attained, regression in the attained milestones were not observed. Initial dose, management protocol for Triac and research into its efficacy on neurodevelopmental signs in MCT8 deficiency are progressing. This case presents evidence that Triac may resolve peripheral thyrotoxicosis successfully and may slow neurodevelopmental regression, while some developmental milestones were achieved after one year of treatment.
Keywords: MCT8 deficiency, Allan-Herndon-Dudley syndrome, triiodothyroacetic acid (Triac), neurodevelopmental outcome, T3 analogue

Introduction

Thyroid hormones (TH) include thyroxine (T4) and triiodothyronine (T3) and are essential for normal physiology, particularly neurodevelopment, and regulation of basal metabolic rate. Precise regulation of intracellular TH signaling by transport of TH across the cell membrane is facilitated by specific hormone transporter proteins, conversion of T4 into T3 or reverse T3 and further degredation into other inactive TH metabolites is regulated by deiodinating enzymes type 1-3, while genomic action of T3 upon binding TH receptors (TR) and, ensures hypothalamus-pituitary-thyroid (HPT) axis homeostasis (1). Monocarboxylate transporter 8 (MCT8), one of these specific membrane transporter proteins, is capable of mediating TH flux through facilitated diffusion (1). While transport of free T3 (fT3) and free T4 (fT4) across the blood-brain-barrier is primarily dependent on MCT8, peripheral tissues rely on other transporters (2,3,4).

Pathogenic variants of *SLC16A2* (located on Xq13.2) that encodes MCT8 cause a rare neurodevelopmental disorder, called MCT8 deficiency or Allan-Herndon-Dudley syndrome (3,4). Multiple hypotheses have been postulated concerning the pathophysiology of the distinct TH fingerprint of elevated serum fT3 concentrations with reduced fT4 and normal thyroid stimulating hormone (TSH) concentrations, hence further studies are warranted (1). It is most likely that impaired TH transport to the central nervous system (CNS) causes the neurodevelopmental findings of severe developmental delay, central hypotonia from birth, and dystonia, as well as hypertonia after the first year of life, while chronic peripheral thyrotoxicosis precipitates tachycardia, muscle wasting, hypermetabolism and progressive weight loss (5,6). The disease has been associated with significant morbidity and mortality (5,6).

Theraputic options for MCT8 deficiency are rather limited but one of these, the natural TH metabolite 3,3',5-triiodothyroacetic acid or tiratricol (Triac), is a suitable candidate. Firstly, as its cellular transport is not dependent on MCT8, it inhibits TSH production and secretion lowering endogenous TH production (7). Secondly it binds to TR1 with a similar affinity to T3 and has a 3- to 6-fold higher affinity than T3 for TR and TR, yet has relatively low thyromimetic activity in peripheral tissues (8). In addition to its effect on the HPT axis, it has been reported that the thyromimetic effect of Triac, at an equal TSH suppressive dose, are at least as potent as those of T4 in most peripheral organs such as liver and skeletal muscle (9). Aiming to resolve both hypothyroidism in the CNS and peripheral hyperthyroidism, clinical studies have indicated that Triac is effective and safe in pediatric patients with MCT8 deficiency when treating peripheral thyrotoxicosis (10). However evaluation of neurocognitive outcome in Triac treated pediatric patients with MCT8 deficiency, especially before completion of brain development and myelination, are lacking (10).

Guiding treatment for rare diseases such as MCT8 deficiency is challenging, as uniform, systematic collection of long-term data are lacking. This case report describes a case of MCT8 deficiency treated with Triac, providing additional clinical evidence about the effect of Triac on peripheral as well as neurocognitive features, while neurodevelopment and myelination continued.

Case Report

A five-month-old boy was referred to the neurology clinic for feeding difficulty, hypotonia and developmental delay. He was born via Cesarian section due to oligohydramnios at 38 weeks of gestation with a birth weight of 2800 grams [-1.1 standard deviation score (SDS)] and a head circumference of 34 cm (-0.5 SDS). He was discharged after a routine follow-up of two days and passed the neonatal hearing test as well as newborn screening tests. He was the sixth child of a healthy Caucasian couple who were second cousins. He had three healthy sisters and two brothers; relevant family history was absent.

Initial physical examination at five months revealed central hypotonia and developmental delay. He was unable to hold his head, smile or make eye contact. Serum TSH was 2.3 mIU/mL with a low fT4 of 10 pmol/L (N = 12-22) (Table 1). Laboratory investigation and metabolic tests were otherwise normal (Table 2). Cranial magnetic resonance imaging revealed hypoplasia of the corpus callosum, and was consistent with delayed myelination. Thyroid function tests suggestive of central hypothyroidism were not present and neurologic follow-up and physiotherapy were implemented. Despite six months of physiotherapy, there was no improvement in physical developmental

milestones and distal muscle tone was increased. Clinical exome sequencing conducted at 12 months for the etiologic investigation of the hypotonic infant revealed a *de novo* novel, hemizygous pathogenic variant (c.430 + 1G > C chrX: 73641903) in *SLC16A2* and MCT8 deficiency was diagnosed (11). The 19-months old patient was then referred to the endocrinology department.

On physical examination, he was underweight (8.1 kg, -2.9 SDS) but his length was 80 cm (-1.3 SDS). Bitemporal narrowing and prominent ears were evident. He was unable to hold his head, smile or make eye contact. Although he had axial hypotonia, deep tendon reflexes were hyperactive and hypertonia in the extremities was evident. Resting tachycardia (148 bpm, 90th-99th percentile) was noted, systolic blood pressure [100/50 mmHg (92nd/84th percentile)]

Table 1. Change in clinical and biochemical signs of MCT8 deficiency on admission (19-months-old), baseline (21-months-old), 6 months after Triac (27-months-old) and 12 months after Triac (33-months-old)

	On admission	Baseline	6 months*	12 months*	Normal range
Auxologic measurements					
Weight (kg)	8.1	8.4	10	11.2	
Weight SDS	-2.9	-2.9	-2.1	-1.8	
Length (cm)	80	82	86	90.2	
Length SDS	-1.3	-1.2	-1.2	-1.1	
Weight for length SDS	-3.2	-3.2	-2.2	-2.1	
Clinical signs of hyperthyroidism					
Resting heart rate (bpm) (percentile)	148 (90th-99th)	150 (90th-99th)	103 (25th-50th)	95 (25th)	
Systolic blood pressure (mmHg)/(percentile)	100 (92nd)	100 (91st)	86 (46th)	90 (56th)	
Diastolic blood pressure (mmHg)/(percentile)	50 (84th)	60 (97th)	54 (87th)	52 (79th)	
Thyroid function tests					
TSH (mIU/mL)	2.83	0.8	0.9	0.7	0.27-4.2
Free T4 (pmol/L)	6.24	5.7	4.4	3.1	12-22
Free T3 (pmol/L)	15.65	11.7	10.9	7.5	3.1-6.8
Laboratory signs of hyperthyroidism					
CK (U/L)	87	168	47	66	< 90
Total cholesterol (mg/dL)	NA	124.7	111.4	128.2	< 170

*Represents time spent from initiation of Triac.
bpm: beats per minute, CK: creatinine kinase, SDS: standard deviation score, MCT8: monocarboxylate transporter 8, TSH: thyroid stimulating hormone

Table 2. Laboratory investigations on admission (19-months-old), baseline (21-months-old), 6 months after Triac (27-months-old) and 12 months after Triac (33-months-old)

	On admission	Baseline	6 months*	12 months*	Normal range
Hemoglobin (g/dL)	11.0	10.8	11.5	10.3	10.7-14.7
Leukocyte (10^3 µ/L)	7.23	7.67	12.8	4.93	7-17.7
Thrombocyte (10^3 µ/L)	311	255	368	277	150-450
BUN (mg/dL)	19.9	23.6	8.9	26.2	5-18
Creatinine (mg/dL)	0.3	0.3	0.2	0.4	0.16-0.39
Albumin (g/L)	3.8	4.1	3.9	4.1	3.8-5.4
Sodium (mEq/L)	136	136.0	136.0	144.0	136.0-146.0
Potassium (mg/dL)	4.3	4.3	4.6	3.9	4.1-5.3
Calcium (mg/dL)	10.2	10.2	9.8	9.5	8.8-10.9
Phosphate (mg/dL)	4.8	4.7	1.8	4.4	3.9-7.7
ALP (U/L)	179	168	232	220	100-450
ALT (U/L)	168	101	77	68	0-40
AST (U/L)	101	130	102	75	0-40
GGT (U/L)	65	55	66	39	0-40

*Represents time spent from initiation of Triac.
BUN: blood urea nitrogen, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamyl transferase

was elevated, but systems were otherwise normal. Endocrine evaluation revealed normal TSH (2.83 mIU/mL), low fT4 (6.24 pmol/L N = 12-22) and increased fT3 (15.65 pmol/L N = 3.1-6.8) (Table 1) with an elevated T3/T4 ratio over 0.75. Laboratory evaluation for signs of peripheral thyrotoxicosis revealed slightly abnormal liver function tests (alanine aminotransferase 168 U/L and aspartate aminotransferase 101 U/L) and creatine kinase (CK) (Tables 1, 2). Total cholesterol was normal (Table 1). Electrocardiography (ECG) and transthoracic echocardiography (TTE) did not reveal any pathological finding.

Triac Treatment

As soon as the diagnosis of MCT8 deficiency was confirmed and required local approvals were completed, Triac (Emcitate tablets, 350 mcg, Egetis Therapeutics, Klara Norra Kyrogata 26, Stockholm, Sweden) was started at 21 months via the compassionate use programme. TH were measured using an immunoassay (Roche Modular E170 Immunology Analyzer, Hague Rd, Indianopolis, USA). The patient was assessed for clinical and biochemical signs of peripheral hyperthyroidism and neurodevelopmental changes, at baseline, 6 months and 12 months after start of treatment with Triac.

Thyroid Function Tests

After an initial dose of 175 mcg/day of Triac, fT3 dramatically decreased to 4.7 nmol/L (N = 3.1-6.8) within two weeks. However it subsequently increased again (Figure 1). An individualized dose escalation of 175 mcg steps with a goal of attaining normal serum fT3 concentration was conducted using frequent clinic visits. After an initial decrease, despite increasing Triac to 1125 mcg/day (twice a day, po), fT3 levels remained high at 8.6-13.1 pmol/L (N = 3.1-6.8) (Figure 1).

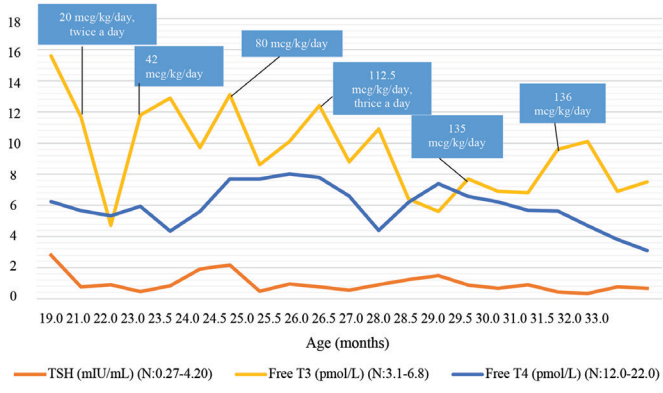

Figure 1. Change in TSH, free T4 and free T3 after initiating Triac when the patient was 21 months old. Blue boxes represent daily Triac dose per kilogram, the dosing regimen was mentioned at the time of change and was continued thereafter

TSH: thyroid stimulating hormone

After identifying adherence issues where there have been times where the patient did not receive treatment for 3-4 consecutive days, the patient was hospitalized for a short time-period and Triac dose adjustment was made. Following discharge, clinic visits included empty Triac box counts in order to try and resolve the adherence issue.

Despite precise treatment, extremely high fT3 (45.1 and 39 pmol/L at the sixth and ninth months of treatment, respectively) were observerd. Concomitant TSH was 0.96 mIU/mL and fT4 5.13 pmol/L (N = 12-22). This unexpected, drastic increase was presumed to be explained by molecular interference of Triac and fT3 in the biochemical analysis, and Triac dose was not changed. In support of this treatment decision, clinical signs of hyperthyroidism were absent and fT3 was near-normal two days after this elevated level (Figure 1). By seven months of treatment, the goal of attaining normal serum fT3 was achieved continuously. Since then, fT3 levels have been high-normal. After one year of treatment, fT3 concentration was reduced to 7.5 pmol/L (N = 3.1-6.8), serum fT4 was 3.09 pmol/L (N = 12-22) and daily dose of Triac was increased eventually to 1500 mcg/day (133 mcg/kg/day) three times a day, po.

Signs of Hyperthyroidism

He was fed orally prior to treatment and continued with oral feedings as consent for tube feeding was not provided by his family. Caloric consumption was adequate for requirements according to age, gender and body weight both prior to and after the treatment. Without change in caloric consumption per kilograms, body weight for length SDS have improved significantly at six months of treatment which was sustained until 12 months afte start of Triac. Resting heart rate measured by ECG, were compared using age adjusted percentile curves and found to be decreased by six months and this was maintained until 12 months (Table 1) (12). Systolic and diastolic blood pressure was decreased and stabilized by 12 months of Triac treatment (Table 1). ECG and TTE were repeated at 12 months and did not reveal any accompanying pathology. Mild elevation in ALT, AST and CK improved within six months (Tables 1, 2). Sex hormone binding globulin could not be measured as it was not available in our clinic.

Problems with adherence to treatment were mainly linked to socioeconomic factors, as well as the populous family structure. No drug related toxicity, need for dose reduction or adverse effect was encountered. He developed upper respiratory tract infection three times during this period which was not attributed to Triac, since they resolved while drug was continued.

Neurodevelopmental Evaluation

Comprehensive developmental assessment was performed by a developmental-behavioral pediatrician. Detailed developmental history and a standardized assessment tool, Bayley Scales of Infant Development 3rd Edition (BSID-III) were used (13). At baseline, prior to Triac (21- months-old) developmental history revealed that developmental milestones were not achieved and BSID-III composite scores for cognitive [55; 95% confidence interval (CI): 51-67], language (47; 95% CI: 43-58) and motor (46; 95% CI: 43-58) evaluation were below two SDS for age.

At six months of Triac treatment, BSID-III composite scores remained below two SDS for age but he started to habituate to objects, recognize and smile at his mother, respond to a person's voice by turning his head, raise his face while in prone position and hold his head steady for a few seconds while in sitting position. As dystonia remained prominent at six months of treatment, oral baclofen was started to ease pain and increase quality of life. At 12 months, BSID-III composite scores remained unchanged (below two SDS for age), but he started to respond to his name by turning his head, follow a person across the room with eyes and head, and hold his head steady for at least 15 seconds and dystonia was improved. Although an improvement in developmental composite scores during follow-up assessments were not observed, an improvement in critical developmental milestones were present. Regression was not observed in any developmental domain.

Discussion

This case highlights the presentation of the extremely rare MCT8 deficiency, which may be missed. Thyroid function tests suggestive of central hypothyroidism in a boy with infantile hypotonia should alert the clinician to measure fT3, and MCT8 deficiency should be in the differential diagnosis, as early recognition and intervention is crucial. This case contributes both peripheral clinical and neurodevelopmental outcomes after one year of Triac treatment and before completion of neurodevelopment and myelination to the literature.

Thorough evaluation of disease presentation and the phenotypic spectrum in MCT8 deficiency, especially concerning neurodevelopmental features, are sparse and disease awareness may be inadequate (6). Data about neurocognitive phenotype and neurodevelopmental outcome are not uniformly collected (5). A natural course study previously described that all patients with MCT8 deficiency had moderate-to-severe intellectual disability with severe delay in motor and language domains (6). At a median age of 7.4 years (0.4-66.8 years), median composite scores in BSID-III were well-below 12 months in all tested sub-domains. In severely affected patients, none of the scores in developmental domains improved with age, while fine motor skills even showed regression.

Triac was shown to decrease serum fT3 concentrations, substantially improving clinical and biochemical signs of hyperthyroidism by six months, which was sustained until 12 months, as in the presented case (5,10). Although Groeneweg et al. (10) reported that 77% of their patients achieved normal serum T3 concentrations by four months of treatment, this only occurred after seven months of treatment in the present case. It may have taken longer in the current patient due to poor adherence, especially in the first months of treatment. The Triac dose (136 mcg/kg/day) required to normalize fT3 was much higher in the present case than previously described (23-48 mcg/kg/day) (8,10). As clinical signs of peripheral hypothyroidism were not observed, it was deduced that this dose compensated the reduction in fT4. While Triac was administered twice or thrice a day in previous studies, we observed that the normal range of fT3 was best maintained if Triac was administered thrice a day (5,10). In addition, clinical signs of hyperthyroidism resolved as progressive deterioration of bodyweight was prevented, and an advancement in bodyweight and length with normal heart rate and blood pressure were accomplished.

Since reduction of fT3 is the aim of successful Triac treatment in MCT8 deficiency, accurate fT3 measurement is crucial for dose adjustment. As T3 and Triac are structurally similar, many commercial total T3 and fT3 assays have been reported to cross-react significantly with Triac in a dose-dependent manner (14). To the best of our knowledge, this is the first clinical case report to address and explain this issue in detail. Chan et al. (14) suggested that patients using Triac should have their T3 hormone monitored using alternative methodologies, such as mass spectrometry. However, as mass spectrometric measurement of thyroid function tests is not available in most institutions such as ours, we propose that MCT8 deficient patients on Triac with unexpected high measurements of fT3 should have their thyroid function test measured by mass spectrometry only if signs of hyperthyroidism are absent.

Previous studies into the efficacy of Triac have demonstrated a notable decrease in serum fT4 (5,6,10). It was speculated that the thyromimetic effect of Triac on peripheral tissues compensated for the reduction in fT4 (10). Since then, the effect of low T4 on neurocognitive function has been a debate and have yet to be clarified. Bárez-López et al. (15) studied this dilemma on MCT8KO mice and concluded that

hypothyroxinemia in the CNS due to low plasma T4 levels may potentially be harmful if this effect was not attenuated by thyromimetic effect of Triac. However, there are some limitations to that study. Firstly, MCT8KO mice' brains were shown to be mildly hypothyroid lacking overt neurological abnormalities and the results of this model on neurocognitive phenotype is not transferrable to humans (1,16). Secondly, the dose of Triac was on the lower side of the recommended dose for humans and dose adjustment was not performed. More recently, MCT8/OATP1C1 DKO mice were validated to be a valuable model organism for the preclinical evaluation of drugs as they exhibit both peripheral and neurocognitive phenotype (17). Previous studies starting Triac as early as possible on the first day of life in MCT8/OATP1C1 DKO mice report a recuperated neuromotor phenotype (18).

Earlier clinical trials on the efficacy of Triac were not designed to detect neurodevelopmental outcomes and study populations' age was very variable (5,10). Although an improvement in developmental composite scores during follow-up were not observed in the present case, clinical improvement in critical developmental milestones were evident, and regression was prevented. At twelve months of treatment, our patient started to respond to his name by turning his head, follow a person across the room with eyes and head, and hold his head steady for at least 15 seconds. Head control, which was previously presented as a marker of improved neurodevelopment and a significant indicator of increased survival rate, was achieved (6). Oral baclofen, a GABA-B agonist was administered for dystonia and related pain. Although the effect of oral baclofen on patients with MCT8 deficiency has not previoulsy been studied, considering it is the most often used oral drug for dystonic cerebral palsy with low efficacy and no known effect on neurodevelopment, oral baclofen was presumed not to have an impact on developmental milestones (19).

Given the insufficiency of data about the effectiveness of Triac in MCT8 deficiency for optimal neurocognitive and neurodevelopmental outcome, larger studies of international collaborative networks for this rare disorder are needed (5). This case presents additional evidence that early diagnosis, while neurodevelopment and myelination continue, is of utmost importance. Early initiation of disease-modifying treatment, Triac, may prevent regression and establish progress in neurodevelopmental milestones while decreasing mortality by its peripheral effects.

Groeneweg et al. (6) reported that overall survival of patients with MCT8 deficiency was greatly diminished with 30% mortality during childhood. Being underweight was associated with increased risk of infections while achieving normal bodyweight was shown to increase median survival from 30.3 to 71 years; however cause of death was unclear in 46.9% (6). It may be hypothesized that mortality due to these unclear reasons and sudden death may be due to a cardiac cause since prevalence of premature atrial and ventricular contractions are high (6). Holter evaluation was not performed in our patient, but cardiac arrythmia was not observed on ECG and improvements in bodyweight, heart rate and blood pressure, as well as markers of thyroid action, may suggest that Triac may decrease the risk factors for mortality in MCT8 deficiency, especially considering the proposals of Groeneweg et al. (6).

Conclusion

The importance of this case is that it reports an in-depth clinical experience with an infant with MCT8 deficiency who was administered Triac. It is essential to keep in mind that fT3 and Triac may cross react if measured with immunoassay in MCT8 deficient patients who are on Triac and thyroid function test should be measured using mass spectrometry if available and if unexpectedly high measurements of fT3 are encountered. Consensus on starting dose of Triac, management protocol and research for its efficacy on central and peripheral signs of MCT8 deficiency are still progressing. This case provides additional evidence that Triac may successfully restore peripheral findings of MCT8 deficiency after one year of treatment. Although a significant change in neurodevelopmental scores were not recorded, neurodevelopmental regression was decelerated and important developmental milestones were achieved. These advances in both peripheral findings of MCT8 deficiency, as well as neurodevelopmental outcomes, may alleviate the risk factors of morbidity as well as mortality.

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Ethics

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: Yağmur Ünsal, Gamze Hayran, Concept: Yağmur Ünsal, Design: Yağmur Ünsal, Data Collection or Processing: Yağmur Ünsal, Gamze

Hayran, Analysis or Interpretation: Yağmur Ünsal, Gamze Hayran, Literature Search: Yağmur Ünsal, Writing: Yağmur Ünsal, Gamze Hayran.

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References

1. van Geest FS, Gunhanlar N, Groeneweg S, Visser WE. Monocarboxylate Transporter 8 Deficiency: From Pathophysiological Understanding to Therapy Development. Front Endocrinol (Lausanne) 2021;12:723750.
2. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, Morte B, Bernal J. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. Endocrinology 2009;150:2491-2496. Epub 2009 Jan 15
3. Chen X, Liu L, Zeng C. A novel variant in SLC16A2 associated with typical Allan-Herndon-Dudley syndrome: a case report. BMC Pediatr 2022;22:180.
4. Visser WE, Vrijmoeth P, Visser FE, Arts WF, van Toor H, Visser TJ. Identification, functional analysis, prevalence and treatment of monocarboxylate transporter 8 (MCT8) mutations in a cohort of adult patients with mental retardation. Clin Endocrinol (Oxf) 2013;78:310-315.
5. van Geest FS, Groeneweg S, van den Akker ELT, Bacos I, Barca D, van den Berg SAA, Bertini E, Brunner D, Brunetti-Pierri N, Cappa M, Cappuccio G, Chatterjee K, Chesover AD, Christian P, Coutant R, Craiu D, Crock P, Dewey C, Dica A, Dimitri P, Dubey R, Enderli A, Fairchild J, Gallichan J, Garibaldi LR, George B, Hackenberg A, Heinrich B, Huynh T, Kłosowska A, Lawson-Yuen A, Linder-Lucht M, Lyons G, Monti Lora F, Moran C, Müller KE, Paone L, Paul PG, Polak M, Porta F, Reinauer C, de Rijke YB, Seckold R, Menevşe TS, Simm P, Simon A, Spada M, Stoupa A, Szeifert L, Tonduti D, van Toor H, Turan S, Vanderniet J, de Waart M, van der Wal R, van der Walt A, van Wermeskerken AM, Wierzba J, Zibordi F, Zung A, Peeters RP, Visser WE. Long-Term Efficacy of T3 Analogue Triac in Children and Adults With MCT8 Deficiency: A Real-Life Retrospective Cohort Study. J Clin Endocrinol Metab 2022;107:1136-1147.
6. Groeneweg S, van Geest FS, Abacı A, Alcantud A, Ambegaonkar GP, Armour CM, Bakhtiani P, Barca D, Bertini ES, van Beynum IM, Brunetti-Pierri N, Bugiani M, Cappa M, Cappuccio G, Castellotti B, Castiglioni C, Chatterjee K, de Coo IFM, Coutant R, Craiu D, Crock P, DeGoede C, Demir K, Dica A, Dimitri P, Dolcetta-Capuzzo A, Dremmen MHG, Dubey R, Enderli A, Fairchild J, Gallichan J, George B, Gevers EF, Hackenberg A, Halász Z, Heinrich B, Huynh T, Kłosowska A, van der Knaap MS, van der Knoop MM, Konrad D, Koolen DA, Krude H, Lawson-Yuen A, Lebl J, Linder-Lucht M, Lorea CF, Lourenço CM, Lunsing RJ, Lyons G, Malikova J, Mancilla EE, McGowan A, Mericq V, Lora FM, Moran C, Müller KE, Oliver-Petit I, Paone L, Paul PG, Polak M, Porta F, Poswar FO, Reinauer C, Rozenkova K, Menevse TS, Simm P, Simon A, Singh Y, Spada M, van der Spek J, Stals MAM, Stoupa A, Subramanian GM, Tonduti D, Turan S, den Uil CA, Vanderniet J, van der Walt A, Wémeau JL, Wierzba J, de Wit MY, Wolf NI, Wurm M, Zibordi F, Zung A, Zwaveling-Soonawala N, Visser WE. Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study. Lancet Diabetes Endocrinol 2020;8:594-605. doi: 10.1016/S2213-8587(20)30153-4. Erratum in: Lancet Diabetes Endocrinol 2020;8:594-605. Erratum in: Lancet Diabetes Endocrinol 2022;10:e7.
7. Senese R, Cioffi F, Petito G, Goglia F, Lanni A. Thyroid hormone metabolites and analogues. Endocrine 2019;66:105-114. Epub 2019 Jul 29
8. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Triiodothyroacetic acid in health and disease. J Endocrinol 2017;234:99-121. Epub 2017 Jun 2
9. Sherman SI, Ringel MD, Smith MJ, Kopelen HA, Zoghbi WA, Ladenson PW. Augmented hepatic and skeletal thyromimetic effects of tiratricol in comparison with levothyroxine. J Clin Endocrinol Metab 1997;82:2153-2158.
10. Groeneweg S, Peeters RP, Moran C, Stoupa A, Auriol F, Tonduti D, Dica A, Paone L, Rozenkova K, Malikova J, van der Walt A, de Coo IFM, McGowan A, Lyons G, Aarsen FK, Barca D, van Beynum IM, van der Knoop MM, Jansen J, Manshande M, Lunsing RJ, Nowak S, den Uil CA, Zillikens MC, Visser FE, Vrijmoeth P, de Wit MCY, Wolf NI, Zandstra A, Ambegaonkar G, Singh Y, de Rijke YB, Medici M, Bertini ES, Depoorter S, Lebl J, Cappa M, De Meirleir L, Krude H, Craiu D, Zibordi F, Oliver Petit I, Polak M, Chatterjee K, Visser TJ, Visser WE. Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: an international, single-arm, open-label, phase 2 trial. Lancet Diabetes Endocrinol 2019;7:695-706. Epub 2019 Jul 31
11. 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
12. Fleming S, Thompson M, Stevens R, Heneghan C, Plüddemann A, Maconochie I, Tarassenko L, Mant D. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. Lancet 2011;377:1011-1018.
13. Bayley N. Bayley scales of infant and toddler development. 3rd ed. San Antonio: Pearson Education, 2006.
14. Chan SL, Refetoff S, Babic N, Jin M, Garg U, Yeo KJ. Triiodothyroacetic Acid Cross-Reacts With Measurement of Triiodothyronine (T3) on Various Immunoassay Platforms. Am J Clin Pathol 2022;157:156-158.
15. Bárez-López S, Obregon MJ, Martínez-de-Mena R, Bernal J, Guadaño-Ferraz A, Morte B. Effect of Triiodothyroacetic Acid Treatment in Mct8 Deficiency: A Word of Caution. Thyroid 2016;26:618-626. Epub 2016 Feb 3
16. Visser WE, Heuer H, Visser TJ. Triiodothyroacetic Acid Treatment in MCT8 Deficiency: A Word of Nuance. Thyroid 2016;26:615-617.
17. Maity-Kumar G, Ständer L, DeAngelis M, Lee S, Molenaar A, Becker L, Garrett L, Amerie OV, Hoelter SM, Wurst W, Fuchs H, Feuchtinger A, Gailus-Durner V, Garcia-Caceres C, Othman AE, Brockmann C, Schöffling VI, Beiser K, Krude H, Mroz PA, Hofmann S, Tuckermann J, DiMarchi RD, Hrabe de Angelis M, Tschöp MH, Pfluger PT, Müller TD. Validation of Mct8/Oatp1c1 dKO mice as a model organism for the Allan-Herndon-Dudley Syndrome. Mol Metab 2022;66:101616. Epub 2022 Oct 18
18. Kersseboom S, Horn S, Visser WE, Chen J, Friesema EC, Vaurs-Barrière C, Peeters RP, Heuer H, Visser TJ. In vitro and mouse studies supporting therapeutic utility of triiodothyroacetic acid in MCT8 deficiency. Mol Endocrinol 2014;28:1961-1970.
19. Monbaliu E, Himmelmann K, Lin JP, Ortibus E, Bonouvrié L, Feys H, Vermeulen RJ, Dan B. Clinical presentation and management of dyskinetic cerebral palsy. Lancet Neurol 2017;16:741-749.