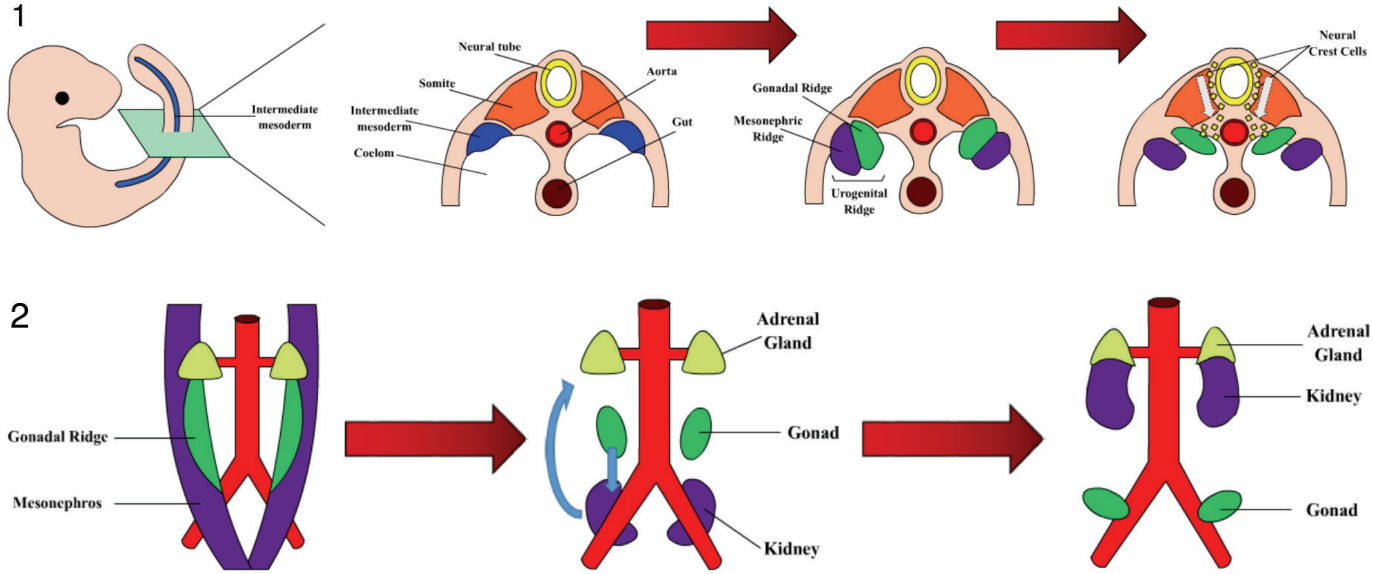
# JCRPE

*Journal of Clinical Research in Pediatric Endocrinology*

September 2022 | volume 14 | issue 3 | www.jcrpe.org | ISSN: 1308-5727

*E-ISSN: 1308-5735*


1. Development of the adrenal cortex and the migration of the neural crest cells to form the adrenal medulla
2. Interaction among the adrenal glands, kidneys and gonads during embryogenesis

Emergence of Ectopic Adrenal Tissues-What are the Probable Mechanisms?

Tarçın G and Ercan O

Page: 258-266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**Date of printing:** August 2022

**ISSN:** 1308-5727 **E-ISSN:** 1308-5735

JCRPE Journal of Clinical Research in Pediatric Endocrinology
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## Review



## Original Articles



## Short Communication



## Case Reports



CONTENTS

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## Letters to the Editor



**CONTENTS**

REVIEW

DOI: 10.4274/jcrpe.galenos.2021.2021.0148

J Clin Res Pediatr Endocrinol 2022;14(3):258-266

# Emergence of Ectopic Adrenal Tissues-What are the Probable Mechanisms?

**Gürkan Tarçın, Oya Ercan**

*İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Pediatric Endocrinology, İstanbul, Turkey*

## Abstract

Ectopic adrenal tissue, defined as the formation of adrenal tissue in an abnormal anatomical location, is not a rare entity and may have clinical significance. Even though the mechanism for their emergence has not been fully understood, numerous cases of ectopic adrenal tissue have been reported, mostly in the vicinity of the original location of adrenal gland, such as in kidneys and gonads. In these cases, most authors attributed their emergence to a probable migration defect. However, this mechanism does not simply explain the ectopic tissues in remote locations, such as in the hypophysis or lungs. This review summarizes these reports, describing many different locations in which ectopic adrenal tissues were encountered, together with their suggested mechanisms.

**Keywords:** Ectopic adrenal, adrenocortical, heterotopia, choristoma, adrenal rest

## Introduction

Embryogenesis begins with a single cell, a newly fertilized oocyte. This cell is totipotent, that is it is capable of giving rise to many different types of tissues, even extraembryonic tissues, such as placenta, under certain physiological conditions. During development from zygote to a multicellular organism, cells are permanently in communication with their neighbor cells, sending and receiving molecular signals. Several intracellular signaling pathways have been elucidated, such as the fibroblast growth factor, sonic hedgehog and wingless pathways. These signaling mechanisms enable the cells to go through an intricate series of events: proliferation, cell fate determination, survival, differentiation, apoptosis, migration, adhesion and cell shape changes (1,2). After the proliferation phase, cells are predisposed to their particular fate and acquire characteristics of different cell lineages and subsequently migrate to their appropriate locations within the embryo. Defects in migration at all stages of development may cause severe conditions (3).

The terms "ectopia" and "heterotopia" are used interchangeably, and ectopic tissues may also be called "accessory" or "rest" tissues. They all refer to the presence of a mature tissue within another tissue due to a developmental anomaly, which occurs when fragments of the tissue split off and move to n inappropraite area or due to an incomplete separation of the tissue from the adjacent organs during development. If heterotopic tissues aggregate and form a tumor-like mass, it is called a "choristoma", as the suffix "-oma" is added to indicate a tumor (4,5). Morgagni, who noticed "yellowish nodules with the characteristics of adrenal tissue in the near vicinity of the gland" was the first to report heterotopic adrenal tissue in 1740 (6). Approximately 150 years later, Marchand (7) encountered adrenal tissue remnants in fetal and infant cadavers, and speculated that adrenal cells could adhere to neighboring tissues and thereby migrate to an abnormal location during early stages of embryogenesis.

## Adrenogenital Development

To better understand the mechanism of emergence of ectopic adrenal tissues, it is necessary to also understand adrenogenital development. The adrenal gland has a dual embryological origin as it consists of two parts: cortex and medulla, arising from the intermediate mesoderm and neural crest cells, respectively. The adrenogonadal primordium appears as a thickening of the coelomic

**Address for Correspondence:** Oya Ercan MD, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Pediatric Endocrinology, İstanbul, Turkey
**Phone:** +90 212 414 30 00 **E-mail:** oyaercan1@gmail.com **ORCID:** orcid.org/0000-0001-7397-2837

**Conflict of interest:** None declared
**Received:** 19.05.2021
**Accepted:** 21.09.2021

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epithelium from about 4 weeks post conception (wpc). Adrenogenital primordium separates from the epithelium and invade the underlying mesenchymal layer of the intermediate mesoderm. The cells next to the mesonephros migrate dorsolaterally to form the gonadal primordium, while the more medial cells migrate dorsomedially to form adrenal primordium at 33 days post conception (dpc). Meanwhile, adrenal medulla begins to develop from the neural crest. From 6 wpc, neural crest cells, which differentiate into chromaffin cells, migrate towards and enter the developing adrenal primordium (Figure 1). By 50-52 dpc, the inner fetal zone, forming the major part, and the outer definitive zone are detectable in the adrenal cortex. By 9 wpc, the adrenal gland is encapsulated and intensely vascularized with subcapsular arteriolar plexus. At this time, the gland contains a cortex containing the definitive zone and fetal zone and a center containing islands of chromaffin cells. A third cortical zone, the transitional zone, found between the definitive zone and the fetal zone, appears by 14 wpc. Subsequently, the fetal zone begins to involute and the definitive zone and transitional zone give rise to the zona glomerulosa and zona fasciculata, respectively, which mature under the stimulation of adrenocorticotropic hormone (ACTH) and angiotensin (8,9,10).

## Ectopic Adrenal Tissues Contain Either Cortex Only or Both Cortex and Medulla

Adrenal rest tissues may contain only cortex or both cortex and medulla, depending on whether the fragments separate before or after medullary tissue migrates into the cortex (5). As mentioned earlier, the adrenal gland, particularly the cortex, has an anatomically close relation to the gonads during embryogenesis. Therefore, it has been postulated that adrenal rests may descend together with the testes in males in the embryological period and contain only cortex, while the adrenal rests close to the normal location of the adrenal gland may also contain medulla. This process has been suggested for and is commonly accepted as the etiology of testicular adrenal rest tumors (5,11).

As for the ectopia of adrenal medulla, chromaffin cells of the adrenal medulla and sympathetic neurons were thought to derive from common neural crest precursors until recently (12). However, recent studies have shown that "Schwann cell precursors" (descendants of the neural crest) directly give rise to adrenal medulla (13). Also, chromaffin cells of the adrenal medulla differ from extra-adrenal chromaffin cells in regard to their "molecular cross-talk" with the steroidogenic cells of the adrenal cortex. This interaction between adrenal chromaffin cells and cortex is essential for the maturation of adrenal medulla, whereas the Zuckerkandl organ, the largest extra-adrenal chromaffin accumulation in mammals, lacks such interaction with steroidogenic tissue and undergoes involution after birth (postnatal autophagy-mediated cell death) in the absence of cortex-derived glucocorticoids (14). This appears to be the reason for the absence of literature describing ectopia of adrenal medulla only, that is not surrounded by cortex, while entities related to adrenal or extra-adrenal chromaffin cells (pheochromocytoma and paraganglioma), and even pheochromocytoma in ectopic adrenal gland have been reported (15,16).

## Probable Impact of Adrenocorticotropic Hormone Levels on the Emergence of Ectopic Adrenal Tissues

Adrenocortical rests are commonly found in children in the retroperitoneal area, appearing as bright-yellow nodules. These tissues are present in approximately half of the newborns and generally undergo atrophy and disappear within several weeks after birth. In some cases adrenocortical rests may also undergo hyperplasia under excess stimulation of increased ACTH secretion, which may even give rise to a neoplasm. The latter mechanism has been suggested in the pathogenesis of testicular adrenal rest tumors in patients with congenital adrenal hyperplasia, and it also explains the higher prevalence of testicular adrenal rests in this patient group (up to 94%) than in healthy newborns (7.5-15%) based on autopsy and surgical findings (17,18). A glucocorticoid replacement regimen for congenital adrenal hyperplasia is useful in the treatment by suppressing ACTH secretion and thereby regulating excess androgen secretion (19).

Suppressing ACTH levels with glucocorticoid replacement has been reported to lead to a reduction in testicular

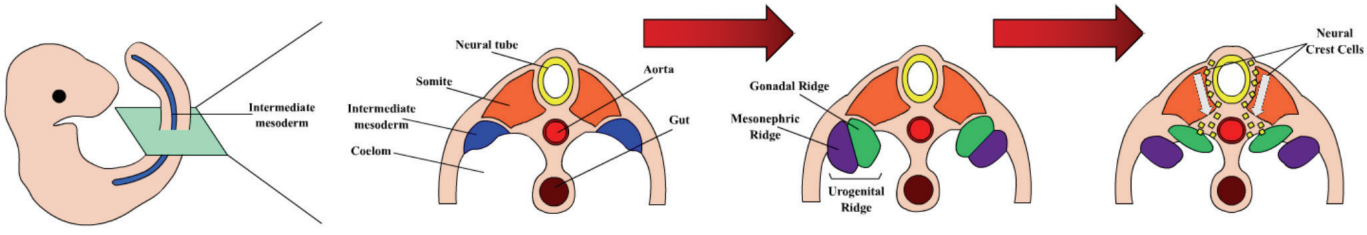


**Figure 1.** Development of the adrenal cortex and the migration of the neural crest cells to form the adrenal medulla

adrenal rest tumor size and improve testicular functions. Concordantly, the prevalence of testicular adrenal rest tumors is higher in patients with severe forms of congenital adrenal hyperplasia who are exposed to higher ACTH levels, even *in utero*, than those with non-classic congenital adrenal hyperplasia with moderate elevation of ACTH, given the fact that ACTH receptors are found in testicular adrenal rest tumor tissues (20).

There are a number of studies and observations supporting the role of ACTH in the development of testicular adrenal rest tumors in patients with congenital adrenal hyperplasia. As with conditions in which patients are exposed to high levels of ACTH from early infancy, adrenal rest tumors have occasionally been reported in Nelson's disease (Table 1) which is an acquired condition in later years of life associated with high levels of ACTH (20).

ACTH plays a crucial role both in the development of adrenal glands and in the regulation of cell differentiation (8). This effect of ACTH on adrenal glands has also been demonstrated in animal research with 12-week-old mice. After an injection of tetracosactide, a synthetic peptide analogue of ACTH, cell proliferation was observed in adrenal zonae glomerulosa and fasciculata of mice, which was consistent with the hypothesis that adrenal cortex may contain progenitor or slow-cycling stem cells, activated by the stimulus of ACTH (21).

High levels of ACTH seem to be a prominent factor for the emergence of adrenal rest tumors. However, these tumors are also found in patients having adequate glucocorticoid replacement and thus have normal levels of ACTH, suggesting that ACTH level is not the only factor involved (22).

## Recent Speculation About Other Possible Stimulating Factors

In recent years, more detailed studies on the origin and characteristics of adrenal rest tumors have demonstrated that these tumors also have Leydig cell characteristics (expression of *INSL3*, *LHCGR* and *HSD17B3* genes) and receptors for angiotensin II and luteinizing hormone, as well as for ACTH. Therefore, it has been hypothesized that these tumors have an origin from a pluripotent cell type, probably from the urogenital ridge or the adrenogonadal primordium, and other factors, such as angiotensin 2 and luteinizing hormone may be involved as well (11,20,22,23,24).

## Defects During the Migration of Precursor Cells

Given that adrenocortical rests are thought to be derived from the urogenital ridge or the adrenogonadal primordium, it is not surprising that these tissues can be found anywhere along the migratory course of the gonads, the descending path of testes and in the vicinity of the kidneys and adrenal glands. Sullivan et al. (25) conducted a study in children under the age of 16 years reporting that in 25 out of 935 groin explorations performed due to various surgical conditions, such as inguinal hernia, undescended testis or hydrocele, adrenocortical tissues were found incidentally in 2.7%. The absence of adrecortical tissue in any of the 35 girls involved in this study supports the idea that adrenocortical tissue fragments may be scattered along the path during the descent of the testes. On the other hand, gonadal adrenal rests are also reported in females in one or both ovaries, broad ligament (26) and fallopian tube (27), though it is rare. These cases were described in adult female patients presenting with heavy menstrual bleeding, dysmenorrhea, progressive

**Table 1. Gonadal masses in patients with Nelson's disease reported in the literature**

| Reference | Gender | Age at diagnosis of Cushing's syndrome | Age at surgery and surgical approach | Interval between surgery and development of ND | Age at diagnosis of the tumor | Location of the tumor |
|---|---|---|---|---|---|---|
| Hamwi et al. (48), 1964 | M | 7 yrs | 7 yrs, left total and right subtotal adrenalectomy | 1 yr | 14 yrs | Left testicle |
| Baranetsky et al. (49), 1979 | F | 24 yrs | 24 yrs, bilateral adrenalectomy | 1 yr | 35 yrs | Bilateral paraovarian |
| Johnson and Scheithauer (50), 1981 | M | 15 yrs | 15 yrs, bilateral adrenalectomy | 1 yr | 23 yrs | Both testicles and left spermatic cord |
| Verdonk et al. (51), 1981 | F | 25 yrs | 25 yrs, bilateral adrenalectomy | 24 yrs | 49 yrs | Bilateral paraovarian and broad ligament |
| Ntalles et al. (52), 1996 | M | 19 yrs | 23 yrs, bilateral adrenalectomy | 1 yr | 29 yrs | Both testicles |
| Puar et al. (24), 2016 | M | 11 yrs | 15 yrs, bilateral adrenalectomy | 1 yr | 27 yrs | Bilateral testes |

ND: Nelson's disease, yrs: years, yr: year, M: male, F: female

abdominal distension, abnormal menstruations and/or menorrhagia (26).

Besides gonadal adrenocortical rests, there are a few case reports describing adrenocortical tissues in the renal hilum (28,29). Both the kidneys and the adrenal cortex arise from mesenchymal tissue, and develop in the pelvis. As the kidneys migrate upwards to the upper lumbar region, they meet the adrenal tissue at 8 wpc. This interaction may explain the underlying mechanism of the adrenocortical tissues in the renal hilum (Figure 2). However, apart from these locations, atypically located adrenal rests (i.e. lungs, hypophysis, spinal canal etc.), the mechanisms of which are more difficult to explain, have also been reported in the literature (5).

## Hypotheses Concerning the Impact of Steroidogenic Factor-1 (NR5A1) Overexpression

In the early 1900s, steroidogenic factor-1 (SF-1) was first identified as a key regulator of steroidogenic enzymes (30). Later, SF-1 was shown to control endocrine development and function, as it is expressed in the adrenal gland, gonads, pituitary gonadotroph cells and ventromedial hypothalamus.

During embryogenesis, the adrenogonadal primordium is identified by its expression of SF-1. Thereafter, the fate of the bipotential precursor cells is determined by their SF-1 expression levels. The cells expressing higher levels of SF-1 form the adrenal primordium, while those expressing lower levels form gonadal primordium (10).

Based on mouse models, deletion of the gene encoding SF-1 was shown to cause agenesis, delayed or incomplete development of the adrenal glands, gonadal dysgenesis, abnormalities of the ventro-medial hypothalamus and partial hypogonadotropic hypogonadism, while overexpression of SF-1 has been shown to cause increased proliferation and decreased apoptosis of the adrenocortical cells, resulting in aberrant proliferation and neoplasia in the adrenal tissue (8,31).

A fetal adrenal enhancer (FaDE) which initiates SF-1 expression has been identified and demonstrated to be activated in gonads, adrenal cortex and, interestingly, in the thorax (32). In a later study conducted with transgenic mice by the same researchers, SF-1 was overexpressed using a FaDE-SF-1 transgene, and in addition to increased adrenal size, emergence of ectopic adrenocortical cells in the thorax expressing steroidogenic markers, including CYP21, was observed. This finding may help explain the few cases of adrenocortical tissues in the thorax. The same study also revealed that in these transgenic mice, gonads remained adjacent to the kidneys. The researchers speculated that emergence of the ectopic adrenal tissue in the inguinoscrotal region resulting from the overexpression of SF-1 could interrupt the separation of the gonads, and attributed this to the frequent accompaniment of cryptorchidism with ectopic adrenal tissue in boys (33).

## Adrenal Rests in the Hypophysis and the Link to SF-1

At the time of writing, five cases of corticotroph adenoma with interspersed adrenocortical cells have been reported (34,35,36,37,38). Of these, four cases were in the pediatric age group. In Table 2, the characteristics and suggested pathogenesis causing ectopia are summarized. Although in three of these cases, the underlying pathogenesis of the misplaced adrenocortical cells was attributed to a migration defect during embryogenesis, the location of the choristoma in the hypophysis is quite distant from those ectopic tissues reported to be in the vicinity of the adrenal gland (35,36,38). Thus, its emergence in the hypophysis is unlikely to stem from a migration defect. As another proposed mechanism, the emergence of the adrenocortical cells interspersed with corticotroph cells was attributed to a possible paracrine mechanism. It was suggested that the corticotroph cells were able to stimulate and convert the neighboring cells to adrenocortical cells (34,37). However, in only one of the five cases (37), corticotroph adenoma was found to be functional (i.e., it had endocrine activity). The most remarkable mechanism was suggested by Mete

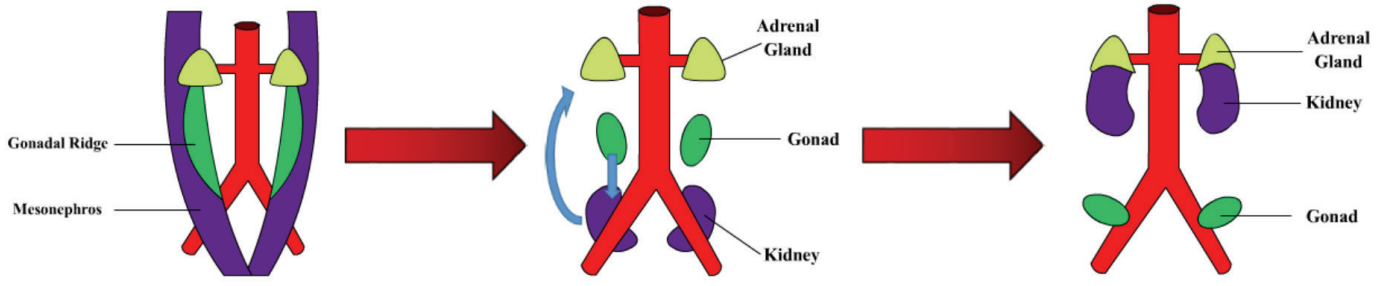


**Figure 2.** Interaction among the adrenal glands, kidneys and gonads during embryogenesis

et al. (36) in 2013, highlighting a common feature between the adrenal and pituitary glands, namely SF-1, which has an important role in the development of the both glands.

SF-1 is a common key molecule in the differentiation of both gonadotropic cells in the hypophysis and adrenocortical cells. Also, in case of SF-1 overexpression, emergence of adrenocortical tissue may be expected, as described earlier. However, the relevant study (33) does not mention a generation of adrenal ectopia in the pituitary region. Also, in addition to SF-1, several other essential regulators, such as Prophet of Pit1 (Prop1), Homeobox protein ANF (HesX1) and Lhx3, play a significant role in the differentiation of gonadotrophs in the hypophysis (39), and differentiation of adrenocortical cells is also dependent on several other regulators, such as DAX1, corticotropin-releasing hormone, ACTH and WT1 (40). Therefore, the hypothesis about an isolated effect of SF-1 appears to be insufficient to explain an erroneous emergence of adrenocortical cells in the hypophysis.

## Unusual Locations of Adrenal Rest Tissues and Proposed Mechanisms

Adrenal rest tissues have also been reported in many places other than the above-mentioned organs, such as in the lung, mediastinum, gastric wall, liver, placenta and spinal canal. While some authors suggested a possible mechanism to explain the abnormal locations of these tissues, others either supported these hypotheses or did not present an opinion at all. The characteristics of the cases and the suggested pathogeneses for the presence of adrenal rest tissues in these abnormal locations are shown in Table 3.

The adrenal rest tissues of all the cases listed in Table 3 involve only cortex, except for the paratracheal 5-mm lesion reported by Shigematsu et al. (41) which contained both cortical and medullary features. Only in three cases adrenal rest tissues were reported to be clinically functional. Two cases presented with signs of virilization (42,43) and one case with Cushingoid appearance (44).

When Table 3 is examined in detail, a few points emerge. In 3 out of 4 cases with adrenal rest tissues located in the placenta, it is noteworthy that the births were premature, and one of the cases had intrauterine growth retardation in addition to being premature. However, the authors did not state if the preterm births could be related to the lesion in the placenta. Only Cox and Chavrier (45) stated that adrenocortical tissue was unlikely to be responsible for intrauterine growth retardation in their report of a late preterm neonate with intrauterine growth retardation. In all the cases of hepatic adrenal rest tissue, the tumors were found in the posterosuperior segment of the right hepatic lobe. As some of the authors of these reports mentioned, hepatocellular carcinomas and adrenal rest tumors cannot be differentiated without an immunohistochemical analysis due to their histological similarity. Therefore, it was suggested that adrenal rest tissues should be considered in the differential diagnosis of the tumors, especially those located in subsegment 7, and immunohistochemical analyses should be performed (46).

Spinal canal is another location where a number of cases of adrenal rest tissues have been reported. Most of the cases were with extramedullary involvement, and presenting symptoms appear to be related to spinal cord compression, such as weakness and/or pain in the legs, lumbar pain or symptoms associated with urination, depending on the affected level of the spine. The access of the adrenal tissue to the spinal canal was suggested to be via the sheath of an exiting nerve or the adventitia of an in-growing segmental lumbar artery of the aorta (47).

## Clinical Features of Adrenal Rest Tissues

Adrenal rest tissues, even if found incidentally in most of the cases, may be symptomatic due to mass effect, as in the case of the spinal canal location. This effect has a

**Table 2. Adrenocortical cells reported within the pituitary gland**

| Reference | Age/gender | Presenting complaint | Tumor size | Suggested pathogenesis |
|---|---|---|---|---|
| Oka et al. (34), 1996 | 16/M | Growth retardation | 15 mm | Possible paracrine effect between two cell types |
| Coiré et al. (38), 1998 | 18/F | Amenorrhea | 13 mm | Abnormal differentiation of a stem cell or misplaced adrenocortical cells during embryogenesis |
| Albuquerque et al. (37), 1999 | 16/M | Delayed growth | 17.5 mm | Possible paracrine interaction |
| Mete et al. (36), 2013 | 35/M | Headache | N/D | Abnormal differentiation of a stem cell which might be related to SF-1 |
| Turan et al. (35), 2021 | 11/M | None, incidental finding on MRI during investigation of central hypothyroidism | 11 mm | Abnormal differentiation of a stem cell or misplaced adrenocortical cells during embryogenesis |

MRI: magnetic resonance imaging, SF-1: steroidogenic factor-1, M: male, F: female, N/D: Not determined

**Table 3. The characteristics of the cases and the suggested pathogeneses for the presence of adrenal rest tissues in other abnormal locations**

| Reference | Age/gender | Presenting complaint | Location/size | Suggested pathogenesis |
|---|---|---|---|---|
| Bozic (53), 1974 | 2-day-old/F | Mild cyanosis after birth (death on the second day) | Superior lobe of the right lung | Misplaced mesothelial cells. As the pleura and adrenal glands have the same mesodermal origin, pleural mesothelium may give rise to ectopic adrenocortical tissue. |
| Armin and Castelli (54), 1983 | 5-day-old/M | Meningitis and midbrain hemorrhage, death on the fifth day | Lung, two nodules measuring 0.5 cm | N/D (Bozic's hypothesis was supported) |
| Shigematsu et al. (41), 2007 | 99 year-old/F | Respiratory disturbance, high fever (Pneumonia) | Paratracheal region/5 mm | Primitive mesothelium and chromophil cells might be incorporated into the ectopic adrenal tissue in the thorax by an unknown mechanism. |
| Medeiros et al. (42), 1992 | 44-year-old/F | Signs of virilization | Mediastinum, in contact with the pericardium/6x5x3.5 cm | N/A |
| Ren et al. (55), 2013 | 72 year-old/F | Upper abdominal discomfort, nausea | Lesser curvature side of the gastric antrum/15x25 mm | Due to the malposition or self-differentiation of mesothelial cells during the embryonic period. |
| Wallace et al. (43), 1981 | 23 year-old/F | Amenorrhea, signs of virilization | Entire right lobe and partially left lobe of the liver/18x15x15 cm | N/A |
| Contreras et al. (44), 1985 | 21/F | Cushing appearance | Posterosuperior subsegment of the right hepatic lobe/4 cm | N/A |
| Arai et al. (56), 2000 | 62/M | None, detected in USG performed for chronic HCV infection | Posterosuperior subsegment of the right lobe of the liver/25x18x15 mm | N/A |
| Tajima et al. (57), 2001 | 55 year-old/F | None, incidentally detected in USG during routine medical check-up | Posterosuperior subsegment of the right hepatic lobe/2.5 cm | Derived from adrenal primordium migrating to neighboring organs |
| Baba et al. (58), 2008 | 67 year-old/F | None, incidentally detected in USG during routine medical check-up | Posterosuperior subsegment of the right hepatic lobe/17x14x11 mm | Derived from adrenal primordium migrating to neighboring organs |
| Shin (59), 2010 | 62 year-old/M | None, incidentally detected in USG during routine medical check-up | Posterosuperior subsegment of the right hepatic lobe/3 cm | N/A |
| Soo et al. (60), 2014 | 47 year-old/F | Elevation of aminotransferases | Segment 7 of the liver/3.4 cm | N/A |
| Valle et al. (61), 2014 | 58 year-old/M | None, found in USG perfomed for HIV and HBV coinfection | Segment 7 of the liver/25 mm | N/A |
| Sugiyama et al. (46), 2015 | 50 year-old/F | Incidentally detected in CT performed for uterine leiomyoma | Segment 7 of the liver/2x3 cm | N/A |
| Enjoji et al. (62), 2017 | 67 year-old/F | None, detected in USG performed for elevated GGT | Segment 7 of the liver/17 mm | Misplaced mesothelial cells or autonomous differentiation of mesodermal elements |
| Cox and Chavrier (45), 1980 | 27 year-old/F | Preterm birth, intrauterine growth retardation | Placenta | During embryogenesis portion of the coelomic mesenchyme migrated, or went astray, and finally became integrated with the extracoelomic mesenchyme within the placenta. |
| Qureshi and Jacques (63), 1995 | 30 year-old/F | Preterm birth, fetal distress, | Placenta | Supported the hypothesis of Cox and Chavrier (45) |
| | 39 year-old/F | Spontaneous rupture of membranes | Placenta | |
| Guschmann et al. (64), 1999 | 29 year-old/F | Spontaneous rupture of membranes, cervical insufficiency, preterm birth | Placenta/2x1 mm | The primordial adrenocortical cells may enter venous vessels during migration. Thus, they might reach the blood vessels within a stem villus of the placenta via shortcut vessels and the umbilical arteries. |

**Table 3. Continued**

| Reference | Age/gender | Presenting complaint | Location/size | Suggested pathogenesis |
|---|---|---|---|---|
| Kepes et al. (47), 1990 | 8 year-old/F | Bilateral leg pain | Spinal canal at the L2 level extramedullary/2.5*1.4*1.3 cm | Cells may have gained access to the spinal canal, perhaps following the sheath of an exiting nerve or the adventitia of an in-growing segmental lumbar artery of the aorta |
| Mitchell et al. (65), 1993 | 16 year-old/F | Intermittent pain in the thigh | Spinal canal at the L1-L2 level extramedullary/1.8*1.5*1.5 cm | N/A |
| | 63 year-old/F | Burning and aching lower back pain | Spinal canal at the L4-L5 level extramedullary/1.5 cm | |
| Cassarino et al. (66), 2004 | 27 year-old/M | Lumbar pain, gait dysfunction, impotence, sphincter dysfunction | Spinal canal, intramedullary/3*2*1.8 cm | N/A |
| Karikari et al. (67), 2006 | 27 year-old/F | Bilateral leg pain, pollacuria | Spinal canal at the L2 level, intramedullary/3.6x2.3x1.1 cm | N/A (But supporting Kepes) |
| Schittenhelm et al. (68), 2008 | 44 year-old/F | Bilateral leg pain | Spinal canal at the L1 level, extramedullary/2.5 cm | N/A |
| Rodriguez et al. (69), 2009 | 5-month-old/F | Weakness in lower extremities | Spinal canal at the T10-L2, extramedullary/6x1.5 cm | N/A |
| Makino et al. (70), 2010 | 17-month-old/F | Inability to crawl and stand | Spinal canal at the T12-L1, L3-L4 extramedullary | N/A |

USG: ultrasonography, CT: computed tomography, M: male, F: female, HBV: hepatitis B virus, HCV: hepatitis C virus, N/A: not available, N/D: not determined

particular importance in testicular involvement in children with congenital adrenal hyperplasia. Even if the testicular adrenal rest tumors are benign, they become hyperplastic and hypertrophic under the stimulation of ACTH. Due to their location in the rete testis, they consequently cause an obstruction of the seminiferous tubules with possible azoospermia and infertility. These tissues have also been demonstrated to secrete adrenocortical hormones, as shown by elevated hormone levels measured from the vein of the tumor compared to the peripheral blood. As already mentioned, there have been a few cases presenting with signs of virilization and Cushingoid appearance, presumably due to the increased levels of these hormones. Briefly, even if adrenal rest tumors are benign, they may become symptomatic due to either their hormonal function, mass effect, or both (17,20).

## Conclusion

Ectopic adrenocortical tissue is not a rare entity, even in the healthy population. However, the mechanism of its emergence is not clearly understood and may differ from location to location, although several mechanisms have been proposed. A migration defect alone could be considered but is unlikely in cases when the ectopic adrenal tissues is distant from the original location. ACTH, with its endocrine or paracrine effect, SF-1 with its effect on stem cells or other as yet unknown factor(s) may also play a role. Further research is required on the subject of how adrenal tissues emerge in all these ectopic locations, given their important clinical consequences.

### Ethics

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: Oya Ercan, Design: Gürkan Tarçın, Oya Ercan, Data Collection or Processing: Gürkan Tarçın, Analysis or Interpretation: Gürkan Tarçın, Oya Ercan, Literature Search: Gürkan Tarçın, Writing: Gürkan Tarçın, Oya Ercan.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther 2019;10:68.
2. Basson MA. Signaling in cell differentiation and morphogenesis. Cold Spring Harb Perspect Biol 2012;4:a008151.
3. Kurosaka S, Kashina A. Cell biology of embryonic migration. Birth Defects Res C Embryo Today 2008;84:102-122.
4. Ozolek JA, Tekkesin MS. THE "-OMAS" and "-OPIAS": Targeted and Philosophical Considerations Regarding Hamartomas, Choristomas, Teratomas, Ectopias, and Heterotopias in Pediatric Otorhinolaryngologic Pathology. Head Neck Pathol 2021;15:25-40. Epub 2021 Mar 15
5. Barwick TD, Malhotra A, Webb JAW, Savage MO, Reznek RH. Embryology of the adrenal glands and its relevance to diagnostic imaging. Clin Radiol 2005;60:953-959.
6. Schechter DC. Aberrant adrenal tissue. Ann Surg 1968;167:421-426.

7. Marchand. Ueber accessorische Nebennieren im Ligamentum latum. Arch für Pathol Anat und Physiol und für Klin Med 1883;92:11-19.
8. Xing Y, Lerario AM, Rainey W, Hammer GD. Development of adrenal cortex zonation. Endocrinol Metab Clin North Am 2015;44:243-274.
9. Kim JH, Choi MH. Embryonic Development and Adult Regeneration of the Adrenal Gland. Endocrinol Metab (Seoul) 2020;35:765-773.
10. Yates R, Katugampola H, Cavlan D, Cogger K, Meimaridou E, Hughes C, Metherell L, Guasti L, King P. Adrenocortical development, maintenance, and disease. Curr Top Dev Biol 2013;106:239-312.
11. Claahsen-van der Grinten HL, Hermus AR, Otten BJ. Testicular adrenal rest tumours in congenital adrenal hyperplasia. Int J Pediatr Endocrinol 2009:624823. Epub 2009 Feb 26
12. Takahashi Y, Sipp D, Enomoto H. Tissue interactions in neural crest cell development and disease. Science 2013;341:860-863.
13. Furlan A, Dyachuk V, Kastriti ME, Calvo-Enrique L, Abdo H, Hadjab S, Chontorotzea T, Akkuratova N, Usoskin D, Kamenev D, Petersen J, Sunadome K, Memic F, Marklund U, Fried K, Topilko P, Lallemend F, Kharchenko PV, Ernfors P, Adameyko I. Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla. Science 2017;357:eaal3753.
14. Kastriti ME, Kameneva P, Adameyko I. Stem cells, evolutionary aspects and pathology of the adrenal medulla: A new developmental paradigm. Mol Cell Endocrinol 2020;518:110998. Epub 2020 Aug 18
15. Bholah R, Bunchman TE. Review of Pediatric Pheochromocytoma and Paraganglioma. Front Pediatr 2017;5:155.
16. Ohsugi H, Takizawa N, Kinoshita H, Matsuda T. Pheochromocytoma arising from an ectopic adrenal tissue in multiple endocrine neoplasia type 2A. Endocrinol Diabetes Metab Case Rep 2019;2019:19-0073.
17. Claahsen-van der Grinten HL, Sweep FC, Blickman JG, Hermus AR, Otten BJ. Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eur J Endocrinol 2007;157:339-344.
18. Savaş C, Candir O, Bezir M, Cakmak M. Ectopic adrenocortical nodules along the spermatic cord of children. Int Urol Nephrol 2001;32:681-685.
19. El-maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. Lancet 2017;390:2194-2210.
20. Engels M, Span PN, van Herwaarden AE, Sweep FCGJ, Stikkelbroeck NMML, Claahsen-van der Grinten HL. Testicular Adrenal Rest Tumors: Current Insights on Prevalence, Characteristics, Origin, and Treatment. Endocr Rev 2019;40:973-987.
21. Chang SP, Morrison HD, Nilsson F, Kenyon CJ, West JD, Morley SD. Cell proliferation, movement and differentiation during maintenance of the adult mouse adrenal cortex. PLoS One 2013;8:e81865.
22. Smeets EE, Span PN, van Herwaarden AE, Wevers RA, Hermus AR, Sweep FC, Claahsen-van der Grinten HL. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. J Clin Endocrinol Metab 2015;100:524-530. Epub 2014 Dec 8
23. Claahsen-van der Grinten HL, Otten BJ, Sweep FC, Span PN, Ross HA, Meuleman EJ, Hermus AR. Testicular tumors in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency show functional features of adrenocortical tissue. J Clin Endocrinol Metab 2007;92:3674-3680. Epub 2007 Jun 26
24. Puar T, Engels M, van Herwaarden AE, Sweep FC, Hulsbergen-van de Kaa C, Kamphuis-van Ulzen K, Chortis V, Arlt W, Stikkelbroeck N, Claahsen-van der Grinten HL, Hermus AR. Bilateral Testicular Tumors Resulting in Recurrent Cushing Disease After Bilateral Adrenalectomy. J Clin Endocrinol Metab 2017;102:339-344.
25. Sullivan JG, Gohel M, Kinder RB. Ectopic adrenocortical tissue found at groin exploration in children: incidence in relation to diagnosis, age and sex. BJU Int 2005;95:407-410.
26. Chew KT, Abu MA, Arifuddin Y, Mohamed Ismail NA, Nasir NAM, Mohammed F, Nur Azurah AG. Ectopic adrenal tissue associated with borderline mucinous cystadenoma of ovary: a case report with review of the literature. Horm Mol Biol Clin Investig 2017;32.
27. Tingi E, Ogah J. Ectopic adrenal rest cells of the fallopian tube: a case report and review of the literature. J Obstet Gynaecol 2018;38:578-579. Epub 2018 Jan 31
28. Tong A, Jia A, Yan S, Zhang Y, Xie Y, Liu G. Ectopic cortisol-producing adrenocortical adenoma in the renal hilum: histopathological features and steroidogenic enzyme profile. Int J Clin Exp Pathol 2014;7:4415-4421.
29. Liu Y, Jiang Y-F, Wang Y-L, Cao H-Y, Wang L, Xu HT, Li QC, Qiu XS, Wang E Liu Y, Jiang YF, Wang YL, Cao HY, Wang L, Xu HT, Li QC, Qiu XS, Wang EH. Ectopic adrenocortical adenoma in the renal hilum: a case report and literature review. Diagn Pathol 2016;11:40.
30. Ferraz-de-Souza B, Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, NR5A1) and human disease. Mol Cell Endocrinol 2011;336:198-205. Epub 2010 Nov 13
31. Ozisik G, Achermann JC, Meeks JJ, Jameson JL. SF1 in the Development of the Adrenal Gland and Gonads. Horm Res 2003;59:94-98.
32. Zubair M, Ishihara S, Oka S, Okumura K, Morohashi K. Two-step regulation of Ad4BP/SF-1 gene transcription during fetal adrenal development: initiation by a Hox-Pbx1-Prep1 complex and maintenance via autoregulation by Ad4BP/SF-1. Mol Cell Biol 2006;26:4111-4121.
33. Zubair M, Oka S, Parker KL, Morohashi K. Transgenic expression of Ad4BP/SF-1 in fetal adrenal progenitor cells leads to ectopic adrenal formation. Mol Endocrinol 2009;23:1657-1667. Epub 2009 Jul 23
34. Oka H, Kameya T, Sasano H, Aiba M, Kovacs K, Horvath E, Yokota Y, Kawano N, Yada K. Pituitary choristoma composed of corticotrophs and adrenocortical cells in the sella turcica. Virchows Arch 1996;427:613-617.
35. Turan H, Tarçın G, Mete Ö, Sinoplu AB, Evliyaoğlu SO, Öz B, Ercan O. Silent corticotroph tumor with adrenocortical choristoma in an eleven-year-old boy. J Clin Res Pediatr Endocrinol 2022;14:126-130. Epub 2021 Feb 15
36. Mete O, Ng T, Christie-David D, McMaster J, Asa SL. Silent corticotroph adenoma with adrenal cortical choristoma: a rare but distinct morphological entity. Endocr Pathol 2013;24:162-166.
37. Albuquerque FC, Weiss MH, Kovacs K, Horvath E, Sasano H, Hinton DR. A functioning composite "corticotroph" pituitary adenoma with interspersed adrenocortical cells. Pituitary 1999;1:279-284.
38. Coiré CI, Horvath E, Kovacs K, Smyth HS, Sasano H, Iino K, Feig DS. A composite silent corticotroph pituitary adenoma with interspersed adrenocortical cells: case report. Neurosurgery 1998;42:650-654.
39. Weltzien FA, Hildahl J, Hodne K, Okubo K, Haug TM. Embryonic development of gonadotrope cells and gonadotropic hormones--lessons from model fish. Mol Cell Endocrinol 2014;385:18-27. Epub 2013 Oct 18
40. Lotfi CFP, Kremer JL, Dos Santos Passaia B, Cavalcante IP. The human adrenal cortex: growth control and disorders. Clinics (Sao Paulo) 2018;73(Suppl 1):473.
41. Shigematsu K, Toriyama K, Kawai K, Takahara O. Ectopic adrenal tissue in the thorax : A case report with in situ hybridization and immunohistochemical studies. Pathol Res Pract 2007;203:543-548. Epub 2007 Jun 27
42. Medeiros LJ, Anasti J, Gardner KL, Pass HI, Nieman LK. Virilizing adrenal cortical neoplasm arising ectopically in the thorax. J Clin Endocrinol Metab 1992;75:1522-1525.

43. Wallace EZ, Leonidas JR, Stanek AE, Avramides A. Endocrine Studies in a Patient with Functioning Adrenal Rest Tumor of the Liver. Am J Med 1981;70:1122-1125.

44. Contreras P, Altieri E, Liberman C, Gac A, Rojas A, Ibarra A, Ravanal M, Serón-Ferré M. Adrenal rest tumor of the liver causing Cushing's syndrome: treatment with ketoconazole preceding an apparent surgical cure. J Clin Endocrinol Metab 2015;60:21-28.

45. Cox JN, Chavrier F. Heterotopic adrenocortical tissue within a placenta. Placenta 1980;1:131-133.

46. Sugiyama T, Tajiri T, Hiraiwa S, Inomoto C, Kajiwara H, Kojima S, Tobita K, Nakamura N. Hepatic adrenal rest tumor : Diagnostic pitfall and proposed algorithms to prevent misdiagnosis as lipid-rich hepatocellular carcinoma. Pathol Int 2015;65:95-99. Epub 2015 Jan 9

47. Kepes JJ, O'Boynick P, Jones S, Baum D, McMillan J, Adams ME. Adrenal cortical adenoma in the spinal canal of an 8-year-old girl. Am J Surg Pathol 1990;14:481-484.

48. Hamwi GJ, Gwinup G, Mostow JH, Besch PK. Activation of Testicular Adrenal Rest Tissue by Prolonged Excessive ACTH Production. J Clin Endocrinol Metab 1963;23:861-869.

49. Baranetsky NG, Zipser RD, Goebelsmann U, Kurman RJ, March CM, Morimoto I, Stanczyk FZ. Adrenocorticotropin-dependent virilizing paraovarian tumors in Nelson's syndrome. J Clin Endocrinol Metab 2015;49:381-386.

50. Johnson RE, Scheithauer B. Massive hyperplasia of testicular adrenal rests in a patient with Nelson's syndrome. Am J Clin Pathol 1982;77:501-507.

51. Verdonk C, Guerin C, Lufkin E, Hodgson SF. Activation of virilizing adrenal rest tissues by excessive ACTH production. An unusual presentation of Nelson's syndrome. Am J Med 1982;73:455-459.

52. Ntalles K, Kostoglou-Athanassiou I, Georgiou E, Ikkos D. Paratesticular tumours in a patient with Nelson's syndrome. Horm Res 1996;45:291-294.

53. Bozic C. Ectopic fetal adrenal cortex in the lung of a newborn. Virchows Arch A Pathol Anat Histol 1974;363:371-374.

54. Armin A, Castelli M. Congenital adrenal tissue in the lung with adrenal cytomegaly. Case report and review of the literature. Am J Clin Pathol 1984;82:225-228.

55. Ren PT, Fu H, He XW. Ectopic adrenal cortical adenoma in the gastric wall: case report. World J Gastroenterol 2013;19:778-780.

56. Arai K, Muro H, Suzuki M, Oba N, Ito K, Sasano H. Adrenal rest tumor of the liver : A case report with immunohistochemical investigation of steroidogenesis. Pathol Int 2000;50:244-248.

57. Tajima T, Funakoshi A, Ikeda Y, Hachitanda Y, Yamaguchi M, Yokota M, Yabuuchi H, Satoh T, Koga M. Nonfunctioning Adrenal Rest Tumor of the Liver : Radiologic Appearance. J Comput Assist Tomogr 2001;25:98-101.

58. Baba Y, Beppu T, Imai K, Masuda T, Iyama KI, Sasano H, Baba H. A case of adrenal rest tumor of the liver: Radiological imaging and immunohistochemical study of steroidogenic enzymes. Hepatol Res 2008;38:1154-1158. Epub 2008 Jun 17

59. Shin YM. Hepatic adrenal rest tumor mimicking hepatocellular carcinoma. Korean J Hepatol 2010;16:338-341.

60. Soo KL, Azhar R, Ooi LL. Hepatic adrenal rest tumour (HART): a case report. Ann Acad Med Singap 2014;43:120-122.

61. Valle RD, Montali F, Manuguerra R, Bresciani P. Adrenal rest tumour of the liver. Dig Liver Dis 2014;46:758. Epub 2014 Apr 3

62. Enjoji M, Sanada K, Seki R, Ito T, Maeda M. Adrenal Rest Tumor of the Liver Preoperatively Diagnosed as Hepatocellular Carcinoma. Case Rep Surg 2017;2017:8231943. Epub 2017 Jun 19

63. Qureshi F, Jacques SM. Adrenocortical Heterotopia in the Placenta. Pediatr Pathol Lab Med 1995;15:51-56.

64. Guschmann M, Vogel M, Urban M. Adrenal Tissue in the Placenta : a Heterotopia Caused by Migration and Embolism? Placenta 2000;21:427-431.

65. Mitchell A, Scheithauer BW, Sasano H, Hubbard EW, Ebersold MJ. Symptomatic intradural adrenal adenoma of the spinal nerve root: report of two cases. Neurosurgery 1993;32:658-661.

66. Cassarino DS, Santi M, Arruda A, Patrocinio R, Tsokos M, Ghatak N, Quezado M. Spinal adrenal cortical adenoma with oncocytic features: report of the first intramedullary case and review of the literature. Int J Surg Pathol 2004;12:259-264.

67. Karikari IO, Uschold TD, Selznick LA, Carter JH, Cummings TJ, Friedman AH. Primary spinal intramedullary adrenal cortical adenoma associated with spinal dysraphism: case report. Neurosurgery 2006;59:E1144.

68. Schittenhelm J, Ebner FH, Harter P, Bornemann A. Symptomatic Intraspinal Oncocytic Adrenocortical Adenoma. Endocr Pathol 2009;20:73-77.

69. Rodriguez FJ, Scheithauer BW, Erickson LA, Jenkins RB, Giannini C. Ectopic Low-grade Adrenocortical Carcinoma Immunohistochemical and Molecular Cytogenetic Study of a Pediatric Case. Am J Surg Pathol 2009;33:142-148.

70. Makino K, Kojima R, Nakamura H, Morioka M, Iyama K, Shigematsu K, Kuratsu J. Ectopic adrenal cortical adenoma in the spinal region: case report and review of the literature. Brain Tumor Pathol 2010;27:121-125. Epub 2010 Nov 3

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2021-10-2

J Clin Res Pediatr Endocrinol 2022;14(3):267-274

# A Long-Term Comparison of Presenting Characteristics of Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic

**Gülay Kaya[1], Emine Ayça Cimbek[2], Osman Yeşilbaş[3], Yusuf Emre Bostan[4], Gülay Karagüzel[2]**

*[1]Karadeniz Technical University Faculty of Medicine, Department of Pediatrics, Trabzon, Turkey*
*[2]Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Turkey*
*[3]Karadeniz Technical University Faculty of Medicine, Department of Pediatric Intensive Care, Trabzon, Turkey*
*[4]Karadeniz Technical University Faculty of Medicine, Department of Public Health, Trabzon, Turkey*

### What is already known on this topic?

The limited use of healthcare services due to fear of Coronavirus disease-2019 (COVID-19) transmission during the pandemic has raised concerns of delays in type 1 diabetes mellitus (T1D) diagnosis, among other diseases. This study investigates the presenting characteristics of newly diagnosed T1D patients assessed in our clinic during the pandemic and compares them with the pre-pandemic period.

### What this study adds?

We observed an increased frequency and severity of diabetic ketoacidosis in children with newly diagnosed T1D in the pandemic period. Our study provides an additional contribution to the literature in its coverage of the one-year period during the pandemic and its comparison with the previous three years.

## Abstract

**Objective:** Diabetic ketoacidosis (DKA) - a potentially preventable complication of type 1 diabetes mellitus (T1D) - is one of the most common chronic childhood diseases, and is associated with a significant risk of morbidity and mortality. The limited use of healthcare services due to fear of Coronavirus disease-2019 (COVID-19) transmission during the pandemic has raised concerns of delays in T1D diagnosis, among other diseases. This study investigated the presenting characteristics of newly diagnosed T1D patients assessed in a single clinic during the pandemic and compares them with the pre-pandemic period.

**Methods:** For the purpose of this study, the first year of the pandemic is referred to as the "pandemic period", and the previous three years as the "pre-pandemic period". Patient files were reviewed retrospectively, the demographic and clinical characteristics and laboratory findings of the patients were recorded, and the findings from both periods were compared.

**Results:** The number of patients diagnosed with T1D in the pandemic period was 44, and in the pre-pandemic period 39 in 2017, 22 in 2018 and 18 in 2019. The two groups had similar age, sex, pubertal stage and anthropometric characteristics ($p > 0.05$). Regarding the type of presentation, the frequency of DKA was significantly higher in the pandemic period (68.2%) than in the pre-pandemic period (40.5%) ($p = 0.006$), and this difference was also observed in the comparison by years ($p = 0.016$). The duration of symptoms ($16.5 \pm 10.7$ vs. $23.5 \pm 17.6$ days) and the length of hospital stay ($10 \pm 3.9$ vs. $15.2 \pm 5.5$ days) were significantly shorter in the pandemic period ($p = 0.032$, and $p < 0.001$, respectively). There was no difference in the frequency of severe DKA between the pandemic (46.7%) and the pre-pandemic (37.5%) periods ($p > 0.05$). However, pH ($7.17 \pm 0.16$ vs. $7.26 \pm 0.14$) and bicarbonate ($12.8 \pm 6.3$ vs. $16.6 \pm 6.3$) levels were significantly lower in the pandemic period ($p < 0.005$). Additional signs of infection on admission were less frequent in the pandemic period (9.1%) than in the pre-pandemic period (27.8%) ($p = 0.027$). The groups did not differ in terms of hemoglobin A1c, C-peptide, concurrent thyroid autoantibodies and tissue transglutaminase antibodies ($p > 0.05$). The rate of anti-glutamic acid decarboxylase positivity was higher in the pandemic period (73.8% vs. 39.2%) ($p = 0.001$) while the frequency of other diabetes-

**Address for Correspondence:** Gülay Karagüzel MD, Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Turkey
**Phone:** +90 462 377 51 80 **E-mail:** gulaykg@yahoo.com **ORCID:** orcid.org/0000-0002-0713-5122

**Conflict of interest:** None declared
**Received:** 11.10.2021
**Accepted:** 10.02.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

J Clin Res Pediatr Endocrinol
2022;14(3):267-274

associated autoantibodies was similar between the groups ($p > 0.05$). The polymerase chain reaction test for COVID-19 was negative in six patients with a history of contact.

**Conclusion:** There was an increased frequency and severity of DKA in children with newly diagnosed T1D in the pandemic period, and these findings justify concerns related to the diagnosis of other diseases during the pandemic. Studies to raise awareness of diabetes symptoms during the pandemic should be continued regularly to reach all segments of society. Our study provides an additional contribution to the literature in its coverage of the one-year period during the pandemic and its comparison with the previous three years.
**Keywords:** COVID-19, type-1 diabetes, diabetic ketoacidosis, child

## Introduction

The novel Coronavirus disease-2019 (COVID-19) caused by the Severe acute respiratory syndrome-Coronavirus-2 virus was first reported in late 2019 in Wuhan in the Hubei province of China (1). The virus spread rapidly all over the world, and the World Health Organization and the Center for Disease Control and Prevention declared COVID-19 a pandemic on March 24, 2020 (2). The first case of COVID-19 in Turkey was reported on March 11, 2020 (3). Most of the hospitals in Turkey were assigned as referral hospitals, so that most were affected by postponement of outpatient appointments. In the first year of the pandemic, Turkey imposed social distancing restrictions from time to time to varying degrees (3). The limited use of healthcare services and the fear of contracting COVID-19 infection in hospitals have led to concerns about delays in the diagnosis of serious diseases.

The early diagnosis of type-1 diabetes mellitus (T1D) and establishment of metabolic control are known to prevent many of the associated acute complications. T1D patients mostly present with symptoms such as polyuria, polydipsia, enuresis and weight loss (4). Diabetic ketoacidosis (DKA) is the most severe, life-threatening, acute complication of T1D. At the onset of newly diagnosed T1D in childhood, the reported frequency of DKA is 15-70% in various regions around the world (5).

The increased presentation of severe DKA among children in the diagnosis of T1D during the COVID-19 pandemic is a major concern. Severe DKA is not only life-threatening but also leads to the use of intensive care beds and resources during a period of potentially high demand. The limited studies of this issue conducted during the pandemic report different findings regarding the frequency of DKA on admission (6,7,8,9,10,11,12,13). The present study compares the clinical and laboratory characteristics of pediatric patients with newly diagnosed T1D during the pandemic with those of the three pre-pandemic years to identify factors affecting the results, and makes recommendations in this regard.

## Methods

This study included newly diagnosed T1D patients aged 0-18 years who were followed up in the Pediatric Endocrinology Clinic and Pediatric Intensive Care Unit of the Karadeniz Technical University Faculty of Medicine. The collected data were analyzed in two groups. For the purpose of this study, the first year of the pandemic (February 1, 2020-January 31, 2021) was considered the "pandemic period" and the previous three years (February 1, 2019-January 31, 2020; February 1, 2018-January 31, 2019; and February 1, 2017-January 31, 2018) were considered the "pre-pandemic period". A retrospective review was made of the file records of the patients, comparing the age and date of diagnosis, sex, family history of diabetes, symptoms on admission to the outpatient clinic (polyuria, polydipsia, enuresis, vomiting, altered consciousness, fever, signs of infection, increased appetite, weight loss, Kussmaul breathing), duration of symptoms, body weight, body mass index (BMI) [BMI = (kg)/height ($m^2$)], pubertal status, venous blood gas [pH, partial pressure of carbon dioxide ($pCO_2$), bicarbonate ($HCO_3$)], hemogram, biochemistry [glucose, blood urea nitrogen, creatinine, corrected sodium, potassium, chloride, amylase, calcium, phosphorus, alkaline phosphatase], hemoglobin A1c (HbA1c), preprandial-postprandial C-peptide, 25-hydroxyvitamin D [25(OH)D], parathormone (PTH), insulin autoantibodies (IAA), islet-cell antibodies (ICA), glutamic acid decarboxylase antibodies (anti-GAD), anti-tissue transglutaminase antibodies, thyroid function tests [tyroid stimulating hormone (TSH), fT3, fT4], anti-thyroid peroxidase (TPO), anti-thyroglobulin (anti-TG) antibodies and length of hospital stay.

HbA1c was measured by spectrophotometric method, and C-peptide levels were estimated by electrochemiluminescence (ECLIA)-(VARIANT II, IMMULITE® 2000 XPi). The presence of IAA, ICA, and GADA was determined by chemiluminescence immunoassay-(SNIBE MAGLUMI). The ECLIA method was used to analyze PTH, 25(OH)D, TSH, fT3, fT4, TPO and anti-TG.

DKA was diagnosed and classified at the time of admission in accordance with the ISPAD 2018 guidelines (5). In contrast

to earlier studies, the present study also included cases with a history and clinical presentation compatible with T1D, but without positive diabetes-associated autoantibodies. Patients with positive IAA, ICA or anti-GAD were classified as type 1a while antibody-negative patients were classified as type 1b. Cases with a random blood glucose value of >200 mg/dL, pH of <7.30 and $HCO_3$ of <15 mmol/L in blood gas, and positive urine ketones in a urine analysis were considered DKA. Patients diagnosed with DKA were divided into mild, moderate and severe groups according to their blood gas results. Cases with a blood gas pH of <7.3 or $HCO_3$ of <15 mmol/L were considered mild DKA, while a pH of <7.2 or $HCO_3$ of <10 mmol/L indicated moderate DKA and a pH of <7.1 or $HCO_3$ of <5 mmol/L indicated severe DKA (5).

The study was initiated upon the written approval by the Clinical Research Ethics Committee of Karadeniz Technical University Faculty of Medicine (protocol number: 2021/210, date: 07.09.2021).

### Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences for Windows, version 23.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics are presented as numbers and percentages for categorical variables, and mean, standard deviation, minimum and maximum for quantitative variables. A Kolmogorov-Smirnov test was used to test the normality of continuous variables; quantitative variables were compared between two independent groups using the Student's t-test or the Mann-Whitney U test; and differences in the rates of categorical variables were analyzed using a chi-square test. The level of statistical significance (alpha) was $p<0.05$. A Fisher's exact test and an ANOVA were used to analyze the differences between the pandemic and pre-pandemic groups.

## Results

In total, the records of 123 newly diagnosed T1D were included in the study. The number of patients with newly diagnosed T1D was higher in the pandemic period (44) than in the three pre-pandemic years (39, 22 and 19, respectively). Demographic, clinical and laboratory data of the groups according to the pre-pandemic-pandemic period and years are presented in Tables 1 and 2. Autoantibody data of pre-pandemic period and pandemic period groups are presented in Table 3. The age, sex, pubertal stage and anthropometric characteristics of the groups were similar ($p>0.05$). Regarding the type of presentation, the frequency of DKA was significantly higher in the pandemic period (68.2%) than in the pre-pandemic period (40.5%) ($p=0.006$), and the difference in the frequency of DKA was also observed in a comparison by year ($p=0.016$). The duration of symptoms (16.5±10.7 vs. 23.5±17.6 days) and the length of hospital stay (10.0±3.9 vs. 15.2±5.5 days) were shorter in the pandemic period ($p=0.032$ and $p<0.001$, respectively). There was no difference in the frequency of severe DKA between the pandemic (46.7%) and the pre-pandemic (37.5%) periods ($p>0.05$). However, the pH (7.1±0.1 vs. 7.26±0.1) and bicarbonate (12.8±6.3 and 16.6±6.3) levels were significantly lower in the pandemic period ($p<0.005$). Additional signs of infection (mostly tonsillitis) on admission were less frequent in the pandemic period (9.1%) than in the pre-pandemic period (27.8%) ($p=0.027$). The groups did not differ in terms of HbA1c, C-peptide, and frequency of concurrent thyroid autoantibodies and tissue transglutaminase antibodies ($p>0.05$). The rate of anti-GAD positivity was higher in the pandemic period (73.8% vs. 39.2%) ($p=0.001$) while the frequency of other diabetes-associated autoantibodies was similar between the groups ($p>0.05$).

Furthermore, 17 of the 40 (42.5%) patients in the pandemic period, and a similar proportion of 28 of the 75 (37.3%) patients in the pre-pandemic period had a 25(OH)D level of <15 µg/L while 23 of the 40 (57.5%) patients in the pandemic period and 47 of the 75 (62%) patients in the pre-pandemic period had a 25(OH)D level of <20 µg/L. Polymerase chain reaction (PCR) tests were performed in six patients with a history of contact, revealing no COVID-19-positive case.

## Discussion

In this study the number of patients with newly diagnosed T1D was higher in the pandemic period than in each of the three pre-pandemic years. The incidence of T1D worldwide has been increasing over the recent years (4). In addition, only one other hospital has a Pediatric Endocrinology Clinic in our city. As that hospital worked as a pandemic hospital during the study period, all patients with T1D were referred to our center, which is the reference hospital. This may explain the higher number of patients during the pandemic period than the pre-pandemic period.

In the present study, significant increases were identified in the frequency (68.2% vs. 40.5%) of children presenting with DKA, the severity of DKA [(pH: 7.17±0.16 vs. 7.26±0.14), and bicarbonate levels (12.8±6.3 vs. 16.6±6.3)] at the onset of newly diagnosed T1D during the COVID-19 pandemic when compared to the pre-pandemic period. While the frequency of severe DKA increased in the pandemic period

when compared to the pre-pandemic period, the difference was not significant (46.7% vs. 37.5%).

The results of other studies conducted during the COVID-19 pandemic regarding DKA and severe DKA in children with T1D at the time of diagnosis are consistent with our findings. A previous study of Canadian children found the presentation of DKA to be higher during the pandemic than in the pre-pandemic period (55% vs. 36.4%), and the same study also established an increased presentation of hospital admissions with severe DKA (48.3% vs. 33.3%) (6). In Australia, a study reported the frequency of DKA in children to be significantly higher during the pandemic than in the pre-pandemic period (73% vs. 26%), and again, the results on the frequency of severe DKA were similar (45%

**Table 1. Demographic, clinical and laboratory results of pre-pandemic and pandemic patients**

| | Pre-pandemic period n = 79 | Pandemic period n = 44 | Total | p |
|---|---|---|---|---|
| Age at T1D onset (years) | 8.13 ± 4.75 | 8.48 ± 4.37 | 8.26 ± 4.60 | 0.572 |
| Sex | | | | 1.000 |
| Female | 46.8% (37) | 45.5% (20) | 46.3% (57) | |
| Male | 53.2% (42) | 54.5% (24) | 53.7% (66) | |
| Pubertal stage | | | | |
| Prepubertal | 69.6% (55) | 59.1% (26) | 65.9% (81) | 0.326 |
| Pubertal | 30.4% (24) | 40.9% (18) | 34.1% (42) | |
| Weight SDS | -0.15 ± 1.32 (79) | -0.10 ± 1.28 (44) | -0.13 ± 1.30 (123) | 0.852 |
| Height SDS | 0.39 ± 1.44 (79) | 0.49 ± 1.12 (44) | 0.42 ± 1.33 (123) | 0.681 |
| BMI SDS | -0.51 ± 1.72 (79) | -0.63 ± 1.55 (44) | -0.55 ± 1.66 (123) | 0.724 |
| Family history | | | | |
| Negative | 81.0% (64) | 79.5% (35) | 80.5% (99) | 1.000 |
| Positive | 19.0% (15) | 20.5% (9) | 19.5% (24) | |
| Duration of symptoms (days) | 23.51 ± 17.60 (79) | 16.54 ± 10.73 (44) | 21.02 ± 15.81 (123) | **0.032** |
| Hospitalization duration (days) | 15.20 ± 5.53 (79) | 10.02 ± 3.89 (44) | 13.35 ± 5.58 (123) | **0.000** |
| Additional infections* | | | | |
| Negative | 72.2% (57) | 90.9% (40) | 78.9% (97) | **0.027** |
| Positive | 27.8% (22) | 9.1% (4) | 21.1% (26) | |
| DKA at presentation | | | | |
| No DKA | 59.5% (47) | 31.8% (14) | 49.6% (62) | **0.006** |
| DKA | 40.5% (32) | 68.2% (30) | 50.4% (61) | |
| Presentations by severity | | | | |
| Mild DKA | 37.5% (12) | 36.7% (11) | 37.1% (23) | 0.662 |
| Moderate DKA | 25.0% (8) | 16.7% (5) | 21.0% (13) | |
| Severe DKA | 37.5% (12) | 46.7% (14) | 41.9% (26) | |
| Creatinine (mg/dL) | 0.56 ± 0.18 (79) | 0.56 ± 0.18 (44) | 0.56 ± 0.18 (123) | 0.920 |
| Calcium (mg/dL) | 9.57 ± 0.57 (79) | 9.51 ± 0.46 (42) | 9.55 ± 0.53 (121) | 0.583 |
| Phosphorus (mg/dL) | 4.33 ± 1.22 (79) | 4.34 ± 0.74 (43) | 4.33 ± 1.07 (122) | 0.626 |
| ALP (U/L) | 245.73 ± 126.40 (74) | 202.65 ± 78.69 (41) | 230.37 ± 113.28 (115) | 0.070 |
| 25(OH)D (µg/L) | 19.00 ± 10.12 (75) | 17.36 ± 9.11 (40) | 18.43 ± 9.77 (115) | 0.538 |
| PTH (ng/L) | 20.44 ± 13.00 (75) | 25.00 ± 15.68 (41) | 22.11 ± 14.14 (112) | 0.101 |
| TSH (mIU/L) | 2.77 ± 1.73 (79) | 2.61 ± 1.74 (43) | 2.71 ± 1.73 (122) | 0.635 |
| fT4 (ng/L) | 1.08 ± 0.32 (79) | 1.03 ± 0.57 (43) | 1.06 ± 0.42 (122) | 0.179 |
| fT3 (ng/dL) | 3.16 ± 0.82 (52) | 3.35 ± 0.99 (36) | 3.24 ± 0.90 (88) | 0.306 |
| pH | 7.26 ± 0.14 (78) | 7.17 ± 0.16 (44) | 7.23 ± 0.15 (122) | **0.002** |
| $PCO_2$ | 31.20 ± 10.88 (78) | 25.95 ± 10.28 (43) | 29.33 ± 10.92 (121) | **0.010** |
| $HCO_3$ (mmol/L) | 16.62 ± 6.30 (78) | 12.77 ± 6.28 (44) | 15.23 ± 6.53 (122) | **0.003** |
| HbA1c (%) | 11.91 ± 2.60 (79) | 12.26 ± 2.57 (43) | 12.03 ± 2.58 (122) | 0.479 |
| Preprandial C-peptide (µg/L) | 0.42 ± 0.40 (77) | 0.81 ± 2.73 (42) | 0.56 ± 1.65 (119) | 0.736 |
| Postprandial C-peptide (µg/L) | 0.68 ± 0.60 (69) | 0.62 ± 0.56 (37) | 0.66 ± 0.58 (106) | 0.464 |

*Additional infections: 70% tonsillitis.
BMI: body mass index, DKA: diabetic ketoacidosis, ALP: alkaline phosphatase, 25(OH)D: 25-hydroxyvitamin D, PTH: parathormone, TSH: tyroid stimulating hormone, $pCO_2$: partial pressure of carbon dioxide, $HCO_3$: bicarbonate, HbA1c: hemoglobin A1c, SDS: standard deviation score, fT3: free triiodothyronine, fT4: free thyroxine

vs. 5%) (7). In a study from Germany, an increase in DKA (23.5% vs. 44.7%) and severe DKA (13.9% vs. 19.7%) were identified during the COVID-19 pandemic, with those under the age of 6 years being at greater risk (8). A study of children in the United Kingdom reported a high rate (70%) of children presenting with DKA during the pandemic, with severe DKA being identified in more than half (52%) (9). In contrast, a study from Poland reported no increase in DKA during the pandemic when compared to the previous year, but identified an increased frequency of severe DKA (10). A multicenter study of children in Saudi Arabia reported a higher rate of DKA in those newly diagnosed with T1D during the pandemic (26%) compared to the pre-pandemic year (13.4%), with the frequency of severe DKA being similar in both years (11). In a study from Italy, fewer pediatric cases of newly diagnosed T1D (23%) but an increased frequency

**Table 2. Demographic, clinical and laboratory results of patients by years**

| | 2017 (n = 39) | 2018 (n = 22) | 2019 (n = 18) | 2020 (n = 44) | Total (n = 123) | p |
|---|---|---|---|---|---|---|
| Age at T1D onset (years) | 7.57 ± 4.40 | 8.52 ± 4.88 | 8.85 ± 5.42 | 8.48 ± 4.37 | 8.26 ± 4.60 | 0.778 |
| Sex | | | | | | 0.848 |
| Female | 51.3% (20) | 45.5% (10) | 38.9% (7) | 45.5% (20) | 46.3% (57) | |
| Male | 48.7% (19) | 54.5% (12) | 61.1% (11) | 54.5% (24) | 53.7% (66) | |
| Pubertal stage | | | | | | 0.355 |
| Prepubertal | 76.9% (30) | 63.6% (14) | 61.1% (11) | 59.1% (26) | 65.9% (81) | |
| Pubertal | 23.1% (9) | 36.4% (8) | 38.9% (7) | 40.9% (18) | 34.1% (42) | |
| Height SDS | 0.17 ± 1.39 | -0.36 ± 1.23 | -0.58 ± 1.16 | -0.10 ± 1.28 | -0.13 ± 1.30 | 0.168 |
| Weight SDS | 0.65 ± 1.20 | 0.11 ± 1.68 | 0.15 ± 1.57 | 0.49 ± 1.12 | 0.42 ± 1.33 | 0.351 |
| BMI SDS | -0.30 ± 1.56 | -0.57 ± 1.60 | -0.90 ± 2.19 | -0.63 ± 1.55 | -0.55 ± 1.66 | 0.636 |
| Family history | | | | | | |
| Negative | 92.3% (36) | 81.8% (18) | 55.6% (10) | 79.5% (35) | 80.5% (99) | |
| Positive | 7.7% (3) | 18.2% (4) | 44.4% (8) | 20.5% (9) | 19.5% (24) | |
| Duration of symptoms (days) | 25.69 ± 20.25 | 20.72 ± 15.97 | 22.22 ± 12.97 | 16.54 ± 10.73 | 21.02 ± 15.81 | 0.143 |
| Hospitalization duration (days) | 14.84 ± 5.69 | 15.59 ± 5.41 | 15.50 ± 5.61 | 10.02 ± 3.89 | 13.35 ± 5.58 | **0.000** |
| Additional infections* | | | | | | |
| Negative | 69.2% (27) | 77.3% (17) | 72.2% (13) | 90.9% (40) | 78.9% (97) | |
| Positive | 30.8% (12) | 22.7% (5) | 27.8% (5) | 9.1% (4) | 21.1% (26) | |
| DKA at presentation | | | | | | |
| No DKA | 66.7% (26) | 50.0% (11) | 55.6% (10) | 31.8% (14) | 49.6% (61) | **0.016** |
| DKA | 33.3% (13) | 50.0% (11) | 44.4% (8) | 68.2% (30) | 50.4% (62) | |
| Presentations by severity | | | | | | |
| Mild DKA | 23.1% (3) | 27.3% (3) | 75.0% (6) | 36.7% (11) | 37.1% (23) | |
| Moderate DKA | 15.4% (2) | 36.4% (4) | 25.0% (2) | 16.7% (5) | 21.0% (13) | |
| Severe DKA | 61.5% (8) | 36.4% (4) | 0.0% (0) | 46.7% (14) | 41.9% (26) | |
| Creatinine (mg/dL) | 0.58 ± 0.18 | 0.55 ± 0.16 | 0.52 ± 0.19 | 0.56 ± 0.18 | 0.56 ± 0.18 | 0.616 |
| Calcium (mg/dL) | 9.54 ± 0.53 | 9.69 ± 0.59 | 9.50 ± 0.64 | 9.51 ± 0.46 | 9.55 ± 0.53 | 0.470 |
| Phosphorus (mg/dL) | 4.31 ± 0.93 | 4.36 ± 0.97 | 4,36 ± 1.93 | 4.34 ± 0.74 | 4.33 ± 1.07 | 0.770 |
| ALP (U/L) | 249.74 ± 131.65 | 260.72 ± 135.25 | 218.05 ± 104.39 | 202.65 ± 78.69 | 230.37 ± 113.28 | 0.191 |
| 25(OH)D (µg/L) | 19.85 ± 10.60 | 18.01 ± 11.71 | 18.30 ± 6.42 | 17.36 ± 9.11 | 18.43 ± 9.77 | 0.598 |
| PTH (ng/L) | 23.32 ± 11.13 | 17.03 ± 17.05 | 19.22 ± 8.95 | 25.00 ± 15.68 | 22.11 ± 14.14 | **0.040** |
| TSH (mIU/L) | 2.81 ± 1.50 | 2.73 ± 2.35 | 2.71 ± 1.38 | 2.61 ± 1.74 | 2.71 ± 1.73 | 0.733 |
| fT4 (ng/L) | 1.13 ± 0.32 | 0.96 ± 0.27 | 1.11 ± 0.35 | 1.03 ± 0.57 | 1.06 ± 0.45 | 0.141 |
| fT3 (ng/dL) | 3.55 ± 0.73 | 2.78 ± 0.84 | 2.91 ± 0.69 | 3.35 ± 0.99 | 3.24 ± 0.90 | **0.018** |
| pH | 7.27 ± 0.15 | 7.22 ± 0.16 | 7.29 ± 0.07 | 7.17 ± 0.16 | 7.23 ± 0.15 | **0.008** |
| $pCO_2$ | 31.12 ± 10.74 | 29.01 ± 11.68 | 33.92 ± 10.20 | 25.95 ± 10.28 | 29.33 ± 10.92 | **0.030** |
| $HCO_3$ (mmol/L) | 17.07 ± 6.58 | 15.16 ± 6.67 | 17.36 ± 5.18 | 12.77 ± 6.28 | 15.23 ± 6.53 | **0.014** |
| HbA1c (%) | 11.74 ± 2.69 | 12.32 ± 2.59 | 11.75 ± 2.50 | 12.26 ± 2.57 | 12.03 ± 2.58 | 0.735 |
| Preprandial C-peptide (µg/L) | 0.43 ± 0.34 | 0.49 ± 0.59 | 0.34 ± 0.23 | 0.81 ± 2.73 | 0.56 ± 1.65 | 0.923 |
| Postprandial C-peptide (µg/L) | 0.71 ± 0.56 | 0.69 ± 0.75 | 0.64 ± 0.49 | 0.62 ± 0.56 | 0.66 ± 0.58 | 0.766 |

*Additional infections: 70% tonsillitis.
BMI: body mass index, DKA: diabetic ketoacidosis, ALP: alkaline phosphatase, 25(OH)D: 25-hydroxyvitamin D, PTH: parathormone, TSH: tyroid stimulating hormone, $pCO_2$: partial pressure of carbon dioxide, $HCO_3$: bicarbonate, HbA1c: hemoglobin A1c, SDS: standard deviation score, fT3: free triiodothyronine, fT4: free thyroxine

of severe DKA were reported during the pandemic when compared to the same period the previous year (44.3% vs. 36%) (12). A study in Turkey reported the rate of DKA in children with newly diagnosed T1D to be higher during the pandemic (91.9%) than in the pre-pandemic year (58.7%), while the frequency of severe DKA was similar in both years (13).

Compared to the pre-pandemic period, the pH and bicarbonate levels on admission were significantly decreased during the pandemic, in line with the increased frequency of DKA. In contrast, there was no statistically significant increase in the frequency of severe DKA. Social isolation and fear of exposure to COVID-19 might have led families to hesitate in referring to hospitals. Although there are currently reduced pandemic restrictions, the pandemic continues, highlighting the significance of our findings.

In newly diagnosed T1D, the risk factors for DKA on admission include age <2 years, ethnic minority, low BMI, delayed diagnosis and low socioeconomic status, while the presence of a first-degree relative with T1D, a parent with a high level of education and a high incidence of T1D in the community are protective factors (14,15,16). No difference in anthropometric characteristics or the presence of T1D in relatives was identified between the pandemic and pre-pandemic groups in the present study, although the duration of baseline symptoms was found to be shorter in the pandemic period than in the pre-pandemic period (16.5±10.7 vs. 23.5±17.6 days). Similarly, Dżygało et al. (10) also identified no significant difference in the family history of T1D between the pandemic and pre-pandemic groups. A previous study from the United Kingdom reported a short duration of symptoms with an increased frequency of severe DKA during the pandemic (9), which may be attributed to the fact that the parents were unaware of the typical symptoms of hyperglycemia during the lockdown restrictions.

Although there is a lack of data about additional infections on admission, the rate of additional infections was lower in the pandemic period than in the pre-pandemic period in the present study (9.1% vs. 27.8%). In Turkey, school education was provided through distance learning during the pandemic, except for certain age groups. Previous studies reported a decrease in the incidence of respiratory syncytial virus, parainfluenza viruses, human metapneumovirus, enteroviruses, adenoviruses and influenza viruses during the pandemic in Europe and Australia when compared to the previous years (17,18). We believe that during the pandemic, children were less exposed to infections due to the need to wear masks, the greater attention paid to hygiene, the limitations on social interactions and the closure of schools during lockdown.

In the present study, the rate of anti-GAD positivity was higher in the pandemic period than in the pre-pandemic period (73.8% vs. 39.2%), and similarly, Dilek et al. (13) reported a higher rate of anti-GAD positivity in the pandemic period than in the pre-pandemic period (45.9% vs. 19.6%). Anti-

**Table 3. Autoantibody results of pre-pandemic and pandemic-period patients**

| | Pre-pandemic period % n = 79 | Pandemic period % n = 44 | Total | p |
|---|---|---|---|---|
| ICA | | | | |
| Negative | 35.4 (28) | 42.9 (18) | 38.0 (46) | 0.546 |
| Positive | 64.6 (51) | 57.1 (24) | 62.0 (75) | |
| Anti-GAD | | | | |
| Negative | 60.8 (48) | 26.2 (11) | 48.8 (56) | **0.001** |
| Positive | 39.2 (31) | 73.8 (31) | 51.2 (62) | |
| IAA | | | | |
| Negative | 87.3 (69) | 95.2 (40) | 90.1 (109) | 0.213 |
| Positive | 12.7 (10) | 4.8 (2) | 9.9 (12) | |
| Anti-TPO | | | | |
| Negative | 87.2 (68) | 88.1 (37) | 87.5 (105) | 1.000 |
| Positive | 12.8 (10) | 11.9 (5) | 12.5 (15) | |
| Anti-TG | | | | |
| Negative | 97.3 (71) | 97.6 (40) | 97.4 (111) | 1.000 |
| Positive | 2.7 (2) | 2.4 (1) | 2.6 (3) | |
| Anti-tTGA | | | | 0.108 |
| Negative | 93.2 (69) | 82.5 (33) | 89.5 (102) | |
| Positive | 6.8 (5) | 17.5 (7) | 10.5 (12) | |
| Type 1b | 26.6 (21) | 14.3 (6) | 22.3 (27) | 0.188 |
| Type 1a | 73.4 (58) | 85.7 (36) | 77.7 (36) | |

ICA: islet cell autoantibody, IAA: insulin autoantibody, anti-GAD: glutamic acid decarboxylase antibodies, anti-TPO: anti-thyroid peroxidase, anti-TG: anti-thyroglobulin, anti-tTGA: anti-tissue transglutaminase antibodies, type 1a: positive diabetes-associated autoantibodies, type 1b: negative diabetes-associated autoantibodies

GAD positivity was found to be a risk factor for autoimmune type-1 diabetes in young children of families exposed to psychosocial stress factors (19). The inability to go to school at all and the limitations on social interactions during the pandemic can be interpreted as psychosocial stress factors. We believe that more detailed studies are required to better clarify the relationship between COVID-19 and anti-GAD positivity.

The length of hospital stay was shorter in the pandemic period than in the pre-pandemic period ($10 \pm 3.9$ vs. $15.2 \pm 5.5$ days), which is a natural consequence of the fact that most hospitals were assigned as referral hospitals, and beds were reserved mostly for COVID-19 patients during the pandemic.

It has been reported that vitamin D deficiency is common at onset of T1D in children (20). In our study, 42.5% of the 40 patients in the pandemic period, and similarly 37.3% of the 75 patients in the pre-pandemic period had 25(OH)D level $< 15$ µg/L and 57.5% of the patients in the pandemic period and 62% of the patients in the pre-pandemic period had a 25(OH)D level $< 20$ µg/L. We found no evidence of increased frequency of vitamin D deficiency or insufficiency associated with inadequate outdoor activities and reduced sunlight exposure due to pandemic restrictions in children with T1D. We could not find any other study examining the frequency of vitamin D deficiency or insufficiency during the COVID-19 pandemic in T1D children.

### Study Limitations

Our work has some limitations and strengths. It adds to the limited existing literature describing DKA frequency in children during the COVID-19 pandemic. Moreover, while most of the studies conducted during the pandemic included short-term (average three-month) comparisons, our study covered a one-year period during the pandemic and compared the findings with those of the previous three years, making an additional longer term contribution to literature. The main limitation of our study is that it is a single-center study. However, our center provides care for all children with T1D over a large geographic region, being a reference center. We also could not perform PCR testing for COVID-19 on all patients participating in the study due to the absence of associated symptoms.

## Conclusion

In conclusion, we observed an increased frequency and severity of DKA in children with newly diagnosed T1D in the pandemic period. Our findings justify the concerns about the diagnosis of other diseases during the pandemic. To enhance physician and social awareness of T1D symptoms during the pandemic, diabetes awareness campaigns should be continued to reach all segments of society.

### Ethics

**Ethics Committee Approval:** The study were approved by the Karadeniz Technical University Faculty of Medicine (protocol number: 2021/210, date: 07.09.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Emine Ayça Cimbek, Osman Yeşilbaş, Gülay Karagüzel, Concept: Gülay Kaya, Gülay Karagüzel, Osman Yeşilbaş, Design: Gülay Kaya, Emine Ayça Cimbek, Yusuf Emre Bostan, Data Collection or Processing: Gülay Kaya, Osman Yeşilbaş, Analysis or Interpretation: Yusuf Emre Bostan, Gülay Kaya, Emine Ayça Cimbek, Gülay Karagüzel, Literature Search: Gülay Kaya, Emine Ayça Cimbek, Yusuf Emre Bostan, Writing: Gülay Kaya, Emine Ayça Cimbek, Gülay Karagüzel.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in wuhan China: the mystery and the miracle. J Med Virol 2020;92:401-402. Epub 2020 Feb 12
2. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, Iosifidis C, Agha R. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID- 19). Int J Surg 2020;76:71-76. Epub 2020 Feb 26
3. Republic of Turkish Ministry of Health Covid-19 Information page. General Coronavirus Table. Last accessed date: 15 Apr 2021. Available from: https://covid19.saglik.gov.tr/EN-69532/general-coronavirus-table.html
4. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, Aschner P, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes 2018;19(Suppl 27):7-19.
5. Wolfsdorf JI, Glaser N, Agus M, Fritsch M, Hanas R, Rewers A, Sperling MA, Codner E. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetic ketoacidosis and the hyperglycemic hyperosmolar state. Pediatr Diabetes 2018;19(Suppl 27):155-177.
6. Sellers EAC, Pacaud D. Diabetic ketoacidosis at presentation of type 1 diabetes in children in Canada during the COVID-19 pandemic. Paediatr Child Health 2021;26:208-209.
7. Lawrence C, Seckold R, Smart C, King BR, Howley P, Feltrin R, Smith TA, Roy R, Lopez P. Increased paediatric presentations of severe diabetic ketoacidosisin an Australian tertiary centre during the COVID-19 pandemic. Diabet Med 2021;38:e14417. Epub 2020 Oct 23

8. Kamrath C, Mönkemöller K, Biester T, Rohrer TR, Warncke K, Hammersen J, Holl RW. Ketoacidosis in Children and Adolescents With Newly Diagnosed Type 1 Diabetes During the COVID-19 Pandemic in Germany. JAMA 2020;324:801-804.
9. Unsworth R, Wallace S, Oliver NS, Yeung S, Kshirsagar A, Naidu H, Kwong RMW, Kumar P, Logan KM. New-Onset Type 1 Diabetes in Children During COVID-19: Multicenter Regional Findings in the U.K. Diabetes Care 2020;43:170-171. Epub 2020 Aug 17
10. Dżygało K, Nowaczyk J, Szwilling A, Kowalska A. Increased frequency of severe diabetic ketoacidosis at type 1 diabetes onset among children during COVID-19 pandemic lockdown: an observational cohort study. Pediatr Endocrinol Diabetes Metab 2020;26:167-175.
11. Alaqeel A, Aljuraibah F, Alsuhaibani M, Huneif M, Alsaheel A, Dubayee MA, Alsaedi A, Bakkar A, Alnahari A, Taha A, Alharbi K, Alanazi Y, Almadhi S, Khalifah RA. The Impact of COVID-19 Pandemic Lockdown on the Incidence of New-Onset Type 1 Diabetes and Ketoacidosis Among Saudi Children. Front Endocrinol (Lausanne) 2021;12:669302.
12. Rabbone I, Schiaffini R, Cherubini V, Maffeis C, Scaramuzza A; Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetes. Has covid-19 delayed the diagnosis and worsened the presentation of type 1 diabetes in children? Diabetes Care 2020;43:2870-2872. Epub 2020 Aug 10
13. Dilek SÖ, Gürbüz F, Turan İ, Celiloğlu C, Yüksel B. Changes in the presentation of newly diagnosed type 1 diabetes in children during the COVID-19 pandemic in a tertiary center in Southern Turkey. J Pediatr Endocrinol Metab 2021;22;34:1303-1309.
14. Vicinanza A, Messaaoui A, Tenoutasse S, Dorchy H. Diabetic ketoacidosis in children newly diagnosed with type 1 diabetes mellitus: Role of demographic, clinical, and biochemical features along with genetic and immunological markers as risk factors. A 20-year experience in a tertiary Belgian center. Pediatr Diabetes 2019;20:584-593. Epub 2019 May 15
15. Szypowska A, Skórka A. The risk factors of ketoacidosis in children with newly diagnosed type 1 diabetes mellitus. Pediatr Diabetes 2011;12:302-306. Epub 2010 Dec 5
16. Usher-Smith JA, Thompson MJ, Sharp SJ, Walter FM. Factors associated with the presence of diabetic ketoacidosis at diagnosis of diabetes in children and young adults: a systematic review. BMJ 2011;343:4092.
17. Tang JW, Bialasiewicz S, Dwyer DE, Dilcher M, Tellier R, Taylor J, Hua H, Jennings L, Kok J, Levy A, Smith D, Barr IG, Sullivan SG. Where have all the viruses gone? Disappearance of seasonal respiratory viruses during the COVID-19 pandemic. J Med Virol 2021;93:4099-4101. Epub 2021 Apr 6
18. Sullivan SG, Carlson S, Cheng AC, Chilver MB, Dwyer DE, Irwin M, Kok J, Macartney K, MacLachlan J, Minney-Smith C, Smith D, Stocks N, Taylor J, Barr IG. Where has all the influenza gone? The impact of COVID-19 on the circulation of influenza and other respiratory viruses, Australia, March to September 2020. Euro Surveill 2020;25:2001847.
19. Sepa A, Wahlberg J, Vaarala O, Frodi A, Ludvigsson J. Psychological stress may induce diabetes-related autoimmunity in infancy. Diabetes Care 2005;28:290-295.
20. Al-Zubeidi H, Leon-Chi L, Newfield RS. Low vitamin D level in pediatric patients with new onset type 1 diabetes is common, especially if in ketoacidosis. Pediatr Diabetes 2016;17:592-598. Epub 2015 Dec 23

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2021-10-14

J Clin Res Pediatr Endocrinol 2022;14(3):275-286

# Hyperinsulinism May Be Underreported in Hypoglycemic Patients with Phosphomannomutase 2 Deficiency

**Doğuş Vurallı[1], Yılmaz Yıldız[2], Alev Ozon[1], Ali Dursun[2], Nazlı Gönç[1], Ayşegül Tokatlı[2], H. Serap Sivri[2], Ayfer Alikaşifoğlu[1]**

*[1]Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Ankara, Turkey*
*[2]Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Metabolism and Nutrition, Ankara, Turkey*

### What is already known on this topic?

Phosphomannomutase 2 deficiency (PMM2-CDG) is an autosomal recessive disorder of protein N-glycosylation with a wide clinical spectrum ranging from life-threatening early-onset multisystem disease to milder phenotypes with isolated neurological involvement. PMM2-CDG is the most common form of congenital disorders of glycosylation.

### What this study adds?

Hypoglycemia is a rare finding in PMM2-CDG, reported in 25 of 1064 patients in the literature (including the three new patients reported here). Half (13/25) of the cases have documented hyperinsulinism which was permanent in two thirds (9/13). Hyperinsulinemic hypoglycemia is usually a part of multisystemic disease with organ involvement. Although insulin levels taken at the time of hypoglycemia may not be very high, hypoglycemia seen in patients with PMM2 still responds well to diazoxide treatment.

## Abstract

**Objective:** Phosphomannomutase 2 deficiency (PMM2-CDG) is a disorder of protein N-glycosylation with a wide clinical spectrum. Hypoglycemia is rarely reported in PMM2-CDG. In this study, we evaluated cause, treatment options and outcomes in cases with hypoglycemia in the course of PMM2-CDG.

**Methods:** Clinical records of patients followed with PMM2-CDG within the last two decades were reviewed. Medical data of patients with hypoglycemia were evaluated in more detail. Demographic and clinical findings, organ involvement and laboratory investigations at time of hypoglycemia were recorded. Time of first attack of hypoglycemia, cause, treatment modalities, duration of hypoglycemia (permanent/transient), and duration of treatment, as well as outcome were also recorded. Other published cases with PMM2-CDG and hypoglycemia are also reviewed in order to elucidate characteristics as well as pathophysiology of hypoglycemia.

**Results:** Nine patients with PMM2-CDG were reviewed, and hypoglycemia was present in three cases. All three had hyperinsulinism as the cause of hypoglycemia. In the first two cases reported here, serum insulin level concurrent with hypoglycemic episodes was elevated, and glucose response was exaggerated during glucagon test, favoring hyperinsulinism. However, in the third case, the serum insulin level at time of hypoglycemia was not so high but hypoglycemia responded well to diazoxide. Hyperinsulinism was permanent in two of these three cases. No genotype-phenotype correlation was observed with respect to hyperinsulinism.

**Conclusion:** The main cause of hypoglycemia in PMM2-CDG appears to be hyperinsulinism. Although insulin levels at the time of hypoglycemia may not be very high, hypoglycemia in patients with PMM2 responds well to diazoxide.

**Keywords:** Congenital disorders of glycosylation, diazoxide, hyperinsulinism, hypoglycemia, phosphomannomutase 2 deficiency

## Introduction

Phosphomannomutase 2 (PMM2) deficiency (PMM2-CDG, formerly named CDG-Ia, OMIM: #212065), which is by far the most common form of congenital disorders of glycosylation (CDG), is an autosomal recessive disorder of protein N-glycosylation with an estimated incidence of 1:20000 (1). It has a wide clinical spectrum, ranging from life-threatening early-onset multisystem disease to milder

**Address for Correspondence:** Doğuş Vurallı MD, Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Ankara, Turkey
**Phone:** +90 312 305 11 24 **E-mail:** dvuralli@hotmail.com **ORCID:** orcid.org/0000-0002-4011-2299

**Conflict of interest:** None declared
**Received:** 30.11.2021
**Accepted:** 28.02.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

phenotypes with isolated neurological involvement. Clinical findings are quite variable, the most common findings being developmental delay, hypotonia and growth retardation (1). During infancy, neurological findings such as strabismus, abnormal eye movements, psychomotor retardation, cerebellar hypoplasia, and hypotonia and hyporeflexia, as well as multisystemic involvement including hepatic disease, chronic diarrhea, cystic renal dysplasia, nephrotic syndrome, hypertrophic cardiomyopathy and pericardial effusion may be observed (2,3,4,5,6). Approximately 20% of cases with visceral involvement die in the first year of life due to multiorgan failure (2,7,8,9,10,11). In addition, dysmorphic findings such as inverted nipples, abnormal distribution of adipose tissue in the supragluteal and suprapubic regions as well as facial dysmorphism, including large and dysplastic ears, are defined in PMM2-CDG (2,12,13,14,15,16,17). During childhood, neurological findings include developmental delay, ataxia, stroke-like episodes, seizures and peripheral neuropathy, as well as retinitis pigmentosa (5). Finally, adults with PMM2-CDG show stable intellectual impairment, stable or progressive ataxia, peripheral neuropathy, progressive kyphoscoliosis and osteoporosis. In addition, the risk of thrombosis is increased due to a decrease in serum levels of several anti-coagulation factors (13).

Most transport proteins and regulators involved in hormonal control are glycosylated so that the endocrine system is one of the main organ systems affected in PMM2-CDG (2). Four functions are especially affected: growth, thyroid function, sexual development and glucose regulation. Glycosylated proteins related to the endocrine system include insulin-like growth factor receptor-binding protein 3, thyroxin binding globulin (TBG), thyroid-stimulating hormone (TSH), prolactin (PRL), follicle-stimulating hormone, and luteinizing hormone. To date, endocrine disorders reported in PMM2-CDG include hyperprolactinemia, short stature despite increased growth hormone release, and hypo- or hyper-gonadotropic hypogonadism (18,19,20,21). Although clinical hypothyroidism is rare, a decrease in TBG and a temporary increase in TSH can be seen in cases with PMM2-CDG (22). Hypoglycemia is also rarely reported, since glycosylated proteins are also involved in glucose homeostasis. In a number of CDGs, including PMM2-CDG, hyperinsulinemic hypoglycemia has been reported. Other forms associated with hyperinsulinemic hypoglycemia are phosphomannose isomerase deficiency (MPI-CDG, CDG1b), phosphoglucomutase 1 (PGM1) deficiency (PGM1-CDG, CDG1t), and alpha-1,3-mannosyltransferase deficiency (ALG3-CDG, CDG1d) (23,24,25). However, data about hyperinsulinemic hypoglycemia in PMM2-CDG is quite sparse.

There is only one recently published systematic review that has collected PMM2-CDG cases with hypoglycemia (26). In this review, it is mentioned that only 23 (2.5%) of 933 cases had hypoglycemia and hyperinsulinemic hypoglycemia was reported as the underlying cause in about half of these cases (10/23). Among the patients with hyperinsulinemic hypoglycemia, clinical data of only seven cases who responded to diazoxide treatment were given. In the remaining 16 cases, neither the cause of hypoglycemia nor the results of the etiological evaluation were reported. There is lack of data in most of the papers mentioning hypoglycemia and little is known about the possible causes, treatment options and consequences of hypoglycemia in PMM2-CDG.

In this study, the cause, treatment options and outcomes in cases with hypoglycemia during the course of PMM2-CDG were evaluated. Other cases with PMM2-CDG and hypoglycemia from the literature are also reviewed in order to elucidate features and characteristics, as well as the pathophysiology of hypoglycemia in PMM2-CDG.

## Methods

Clinical records of patients followed with PMM2-CDG within the last two decades at one of the largest tertiary medical centers for inborn errors of metabolism in Turkey, were reviewed retrospectively for demographic, clinical, laboratory, imaging and molecular genetic findings. All patients were diagnosed by type 1 pattern on capillary zone electrophoresis of serum sialotransferrins (decreased tetrasialo- and increased disialo- and asialo-transferrin), followed by Sanger sequencing of the *PMM2* gene. Medical data of patients with hypoglycemia were evaluated in more detail. The demographic and clinical findings including sex, age at onset of the disease, age at diagnosis, age at the last follow-up, organ involvements, and specific findings of the organ systems, as well as the results of the laboratory examinations at the time of hypoglycemia including serum glucose, insulin, cortisol, growth hormone levels, and urinary ketones were recorded. Time of the first attack of hypoglycemia, cause, treatment modalities, duration of hypoglycemia (permanent/transient), duration of treatment, and outcome were also recorded. If the patient died, the age and cause of death were documented. The NCBI reference sequence NM_000303.3 was used for reporting *PMM2* gene variants.

We also performed a literature search in PubMed using the following search terms: carbohydrate-deficient glycoprotein syndrome OR CDG-Ia OR CDG type Ia OR PMM2 deficiency OR PMM2-CDG OR PMM2-CDG AND hypoglycemia OR

hyperinsulinism OR hyperinsulinemia. We searched the literature from inception to the end of December 2020. Only the PMM2-CDG cases confirmed by enzymatic or molecular genetic analysis and who were reported to have hypoglycemia were included in the review.

### Statistical Analysis

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). Normality was tested using the Shapiro-Wilk test. Continuous data are reported as mean ± standard deviation and categorical data are reported 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 using one-way ANOVA.

## Results

Nine patients who had been diagnosed with PMM2-CDG were included in the study, eight of whom had been previously reported by Yıldız et al. (27). Hypoglycemia was present in three cases.

### Case 1

The male patient was referred at the age of 5 months because of chronic diarrhea, vomiting, seizures and failure to thrive. He was born at the 36$^{th}$ week of gestation as the second child of healthy, non-consanguineous parents with a birth weight of 2.8 kg [0.2 standard deviation score (SDS)]. He was hospitalized in the neonatal intensive care unit due to prematurity, respiratory distress syndrome and neonatal hypoglycemia. He had mild hypothyroxinemia and hyperthyrotropinemia [fT4: 11.2 pmol/L (N: 12-22), TSH: 8.7 µIU/mL (N: 0.27-4.2)], and was started on Na-L-thyroxine. At the time of referral, weight was 4 kg (-4.1 SDS, when corrected for gestational age -3.5 SDS), height 59 cm (-2.6 SDS, -1.7 SDS when corrected for gestational age) and head circumference 37 cm (-4.2 SDS, -3.5 SDS when corrected for gestational age). He had dysmorphic facies, microcephaly, sensorineural hearing loss, inverted nipples, abnormal fat distribution, axial hypotonia and a hepatomegaly of 2 cm below the costal margin. In addition to feeding difficulties, he had vomiting, and chronic diarrhea. Investigations revealed hypoalbuminaemia, hypertransaminasemia, hypertriglyceridemia, ascites, increased renal echogenicity, pericardial effusion and cardiac hypertrophy. He had low blood glucose levels (<50 mg/dL) and an intravenous (iv) glucose infusion was given at a rate of 6-10 mg/kg/min. Hypoglycemia recurred when the glucose infusion rate was reduced. A critical sample during hypoglycemia (serum glucose 46 mg/dL) revealed elevated insulin and c peptide levels [6.0 µIU/mL (N: <1), and 9 ng/mL (N: <0.9) respectively] suggesting hyperinsulinemia. Simultaneous cortisol (35 µg/dL, N: >20) and growth hormone (19.6 ng/mL, N: >10) excluded adrenal insufficiency and growth hormone deficiency, respectively. Lactic acid (12 mg/dL; N: 4.5-19.8) and ammonia (46 µg/dL, N: 20-120) were normal, while urinary ketones were negative. Tandem mass spectrometry, plasma and urinary amino acid profiles, and urinary organic acid analyses were normal. A glucagon test during hypoglycemia showed an exaggerated glucose response (0, 15 and 30 min glucose levels: 35, 70, and 85 mg/dL, respectively) supporting hyperinsulinism. The patient died due to complications of epilepsia partialis continua before diazoxide could be initiated. Transferrin isoform electrophoresis for a preliminary diagnosis of CDG was abnormal with a type 1 pattern.

### Case 2

The female patient was referred to our hospital at the age of 8 months with the complaints of poor feeding, failure to thrive and strabismus. She was born at term as the first child of healthy, non-consanguineous parents with a birth weight of 2.7 kg (-1.3 SDS). Her parents noticed failure to thrive and strabismus when she was 2 months old, however a specific diagnosis was not available. On admission to our hospital, she was underweight (6 kg, -2.5 SDS) with short stature (63 cm, -1.9 SDS) and microcephaly (41 cm, -2.0 SDS). She had abnormal eye movements, dysmorphic facies, inverted nipples, supragluteal fat pads, orange peel skin, axial hypotonia, and a hepatomegaly of 3 cm below the costal margin. Ophthalmological examination revealed internal strabismus and normal fundoscopy. There was no history of seizures, but she had hypotonia, hyporeflexia and developmental delay. She was fed by nasogastric feeding since she had swallowing difficulties. Feeding intolerance due to abdominal distention, and respiratory distress due to transient pleural effusion limited her oral and enteral intake. Biochemical investigations revealed hypoalbuminaemia, hypertransaminasemia, and hypertriglyceridemia, low high density lipoprotein (HDL) levels, and hypocholesterolemia. She had severe hypoglycemia (blood glucose <40 mg/dL) and iv glucose infusion was administered at a rate of 8-12 mg/kg/min. Again, a critical blood samples taken during an episode of hypoglycemia (serum glucose was 36 mg/dL) revealed an insulin of 3.1 µIU/mL (N: <1), c-peptide 1 ng/mL (N: <0.9), cortisol 6.19 µg/dL (N>20), adrenocorticotropin hormone (ACTH) 25.8 pg/mL (N: 0-46), growth hormone 8.01 ng/mL (N>10), lactic acid 14 mg/dL (N: 4.5-19.8), ammonia 33 µg/dL (N: 20-120), and urinary ketone was negative. Tandem mass spectrometry, plasma and urinary

amino acid profiles, and urinary organic acid analyses were normal. Low dose ACTH stimulation was normal, ruling out central adrenal insufficiency as the cause of hypoglycemia. Hyperinsulinism was considered, since the levels of insulin and c-peptide were not suppressed appropriately during hypoglycemia, and an exaggerated glucose response was observed during glucagon test (0, 15 and 30 min glucose levels: 36, 84, and 95 mg/dL, respectively). The patient was started on diazoxide at a dose of 10 mg/kg/day, and experienced no new episodes of hypoglycemia after diazoxide.

Further analyses that led to the diagnosis of PMM2-CDG were: prolonged prothrombin and activated prothrombin times; decreased antithrombin 3 and factor 11 levels; ascites; increased myocardial, hepatic and renal echogenicity; proteinuria; pleural and pericardial effusion; cardiac hypertrophy; cerebellar atrophy and diffuse volume loss in the brainstem. Minimally elevated serum TSH (5.6 µIU/mL) with normal free T4 level was detected in the examinations performed in terms of other endocrinological disorders that may accompany PMM2-CDG. Serum PRL level was normal. Serum IGF1 and IGBP3 levels were 5.6 ng/mL (-4.5 SDS) and 1530.8 ng/mL (-0.1 SDS), respectively. Transferrin isoform electrophoresis was abnormal with a type 1 pattern.

Around the time of discharge, her feeding improved and she was able to tolerate more enteral feeds, allowing the tapering and discontinuation of diazoxide after six months. At the age of four years she has global, but stable developmental delay. She requires intermittent transfusions for thrombocytopenia. Transaminase levels are elevated without clinical hepatic manifestations. Glucose levels are stable with frequent meals and continuous enteral feeding at night, without need for diazoxide.

### Case 3

The male patient was referred to our hospital at the age of 2 months with the complaints of chronic diarrhea, vomiting, and failure to thrive. He was born at the 36$^{th}$ week of gestation as the second child of healthy, non-consanguineous parents with a birth weight of 2.1 kg (-2.3 SDS). He was hospitalized for 18 days due to prematurity, pneumonia and meconium aspiration syndrome. In follow-up he was diagnosed with primary congenital hypothyroidism and Na-L-thyroxine treatment was started [fT4: 6.1 pmol/L (N: 12-22), TSH: 100 µIU/mL (N: 0.27-4.2)]. He also had an afebrile seizure in the neonatal period but the etiology was not known. On admission at the age of 2 months, he was 3.1 kg (-3.3 SDS, when corrected for gestational age -2.0 SDS) in weight, 50 cm (-3.4 SDS, when corrected for gestational age -1.9 SDS) in height and had a head circumference of 35 cm (-2.8 SDS, when corrected for gestational age -1.6 SDS). He had dysmorphic facies, microcephaly, sensorineural hearing loss, inverted nipples, generalized edema and hypotonia. The eye examination was normal. He had anemia with a normal thrombocyte count. He also had hypoglycemia, hypoalbuminaemia, proteinuria, hypertransaminasemia, hypocholesterolemia, decreased levels of coagulation and anti-coagulation factors, increased renal echogenicity, and mild right ventricular hypertrophy. In subsequent hospitalizations, he developed pericardial effusion necessitating pericardiocentesis, and deep venous thrombosis at sites of central venous catheters.

He had hypoglycemia and an iv glucose infusion was administered at a rate of 6-10 mg/kg/min. Hypoglycemia recurred when the iv glucose infusion rate was reduced. Serum insulin level was 1.7 µIU/mL (N: < 1) and c-peptide was 1.5 ng/mL (N: < 0.9) when serum glucose was 38 mg/dL. Concurrently measured cortisol level was 11.5 µg/dL (N > 20), growth hormone 38 ng/mL (N > 10), lactic acid 7.3 mg/dL (N: 4.5-19.8), ammonia 32.9 µg/dL (N: 20-120), and urinary ketones were negative. Tandem mass spectrometry, plasma and urinary amino acid profiles, and urinary organic acid analyses were normal. Growth hormone deficiency was excluded with high levels of growth hormone during hypoglycemia. Cortisol deficiency was excluded with a peak cortisol level of 20.6 µg/dL during low dose ACTH stimulation test. His other blood glucose levels and simultaneously taken insulin and c-peptide levels were: glucose 46 mg/dL; insulin 1.7 µIU/mL; c-peptide 1.2 ng/mL; and glucose 43 mg/dL; insulin 1.9 µIU/mL; c-peptide 1.0 ng/mL, respectively. Urinary ketones were negative during all hypoglycemic episodes. Hypoglycemia due to energy deficiency and/or storage deficiency could not be excluded since he had a low birth weight and thus his daily caloric, protein, carbohydrate and lipid intake was increased. The patient could not take the required calories orally so he was fed with nasogastric drip infusion. Insulin values obtained at the time of hypoglycemia were high and measurable ( > 1 µIU/mL) favoring hyperinsulinism, and since nonketotic hypoglycemia was persistent despite having a diet with enough calories, diazoxide was started at a dose of 10 mg/kg/day. His blood glucose levels normalized after the beginning of diazoxide treatment. Transferrin isoform electrophoresis, requested following a preliminary diagnosis of CDG was abnormal with a type 1 pattern.

### Molecular Analyses

The *PMM2* gene sequencing revealed compound heterozygous c.422G > A (p.Arg141His)/c.691G > A (p.Val231Met) variants in Case 1, homozygous c.385G > A

(p.Val129Met) variant in Case 2, and compound heterozygous c.349G > T (p.Gly117Cys)/c.359T > C (p.Ile120Thr) variants in Case 3 (RefSeq NM_000303.3). The parents were heterozygous for one allele each, consistent with autosomal recessive inheritance. All of these variants, except for c.349G > T (p.Gly117Cys) have been previously reported in patients with PMM2-CDG (28). The c.349G > T (p.Gly117Cys) missense variant has a low allele frequency in the healthy population, is predicted to be pathogenic by multiple lines of computational evidence, and is located in a dense hot-spot where a different amino acid change (p.Gly117Arg) has been reported as pathogenic and is thus classified as pathogenic according to the American College of Medical Genetics and Genomics 2015 criteria (28,29).

### Literature Review

Hypoglycemia was reported in 37 cases (3.4%) among a total of 1.060 patients with PMM2-CDG in 171 articles in PubMed. However, in a few of these articles, only the number of hypoglycemic cases was reported without any further detail (30,31). Therefore, only data of 22 cases with hypoglycemia in whom clinical and laboratory features are published could be included in the current analysis. The clinical and laboratory findings related to hypoglycemia, as well as management and outcome of hypoglycemic patients with PMM2-CDG, are shown in Table 1. Hypoglycemia occurred in the first year of life in all cases, and in the early months of life in most cases. Hypoglycemia was one of the main presenting findings in six of the cases. Symptoms of hypoglycemia included seizures, decreased consciousness, and poor feeding. Blood glucose levels were between 9 and 50 mg/dL. Hyperinsulinism was the cause of hypoglycemia in approximately 45% (10/22) of the cases, and the cause of hypoglycemia was not specified in the remainder. Serum insulin levels ranged between 8.3 and 33 pmol/L (1.2 and 4.8 µIU/mL) at the time of hypoglycemia. Serum levels of ACTH, cortisol, growth hormone, and growth factors, which could clarify the cause of hypoglycemia were not specified in any of the cases, except one. Only two patients with hyperinsulinemic hypoglycemia had documentation of negative serum ketone levels, whereas ketone levels were not reported in others. A patient with hyperinsulinemic hypoglycemia had abdominal magnetic resonance imaging and no pathology was found in the pancreas (19). Abdominal ultrasonography was available in four of the cases. Normal pancreas anatomy was observed on ultrasonography in one patient in whom etiology of hypoglycemia was not specified, and in two hyperinsulinemic cases, while a cystic lesion on the head of the pancreas was detected in a hyperinsulinemic patient who had recurrent attacks of acute pancreatitis (19,32,33). Pancreatic biopsy was performed in a patient with hyperinsulinemic hypoglycemia who had normal abdominal ultrasonography, and histological examination revealed pancreatic islets cells of normal size and distribution with hypertrophic nuclei in a few beta cells (32). Hypertrophic nuclei in beta cells were evaluated as sign of functional activity.

A wide spectrum of multisystem involvement was present in cases with hypoglycemia. The specific organ involvements of all the cases with hypoglycemia are shown as supplementary material (Supplementary Table 1). Of all hypoglycemic cases, 73% had failure to thrive, 68% had inverted nipples, 64% had abnormal fat distribution, 55% had internal strabismus, 41% had dysmorphic facies, and 32% had microcephaly. Cardiac involvement (pericardial effusion and cardiac hypertrophy) was present in 59% of the cases, and gastrointestinal system involvement (feeding difficulties, vomiting, gastroesophageal reflux, gastrointestinal dysmotility, and chronic diarrhea) in 73%. Liver involvement, such as hypertransaminasemia and hepatomegaly, was present in 64% of all hypoglycemic patients, kidney involvement, such as proteinuria and tubulopathy, in 64%, coagulation problems, such as coagulation factor deficiencies, bleeding diathesis and thrombosis in 55%, and central nervous system involvement, such as hypotonia, developmental delay, cerebellar hypoplasia, hyporeflexia, seizures and ataxic gait, in 77%.

Some of the patients with hypoglycemia had severe progressive neurological, cardiovascular, hepatic, and gastrointestinal system involvement. Some had mild involvement of different organ systems. In some cases, skeletal findings, such as scoliosis, rhizomelia, generalized epiphyseal ossification delay, ovoid and anteriorly beaked vertebral bodies, bullet shaped short tubular bones of the hand, joint hyperlaxity, arachnodactyly, spinal canal stenosis, hypoplastic cervical vertebrae, thirteen pairs of costae, and shortening of long bones were also reported. Pseudocholinesterase deficiency and multiple episodes of sepsis were shown in one case. Other accompanying findings included thrombocytopenia, fetal hydrops, acute pancreatitis, stroke-like episodes, peripheral neuropathy, and athetosis. Hyperinsulinism was the main problem in one case and this case did not have other serious multisystem organ involvements (19).

When the cases are examined in terms of accompanying endocrinological disorders, thyroid dysfunction was reported in 10 (10/22, 45%) of the hypoglycemic cases, it was stated that thyroid function was normal in one case, and information about the thyroid function of the other cases was not provided. It was reported that TBG and serum T4 values

**Table 1. Clinical and laboratory findings related to hypoglycemia as well as management and outcome of hypoglycemic patients with PMM2-CDG**

| Author, year published, reference | Antoun et al. (33) 1999 | Böhles et al. (32) 2001 | Enns et al. (15) 2002 | Enns et al. (15) 2002 | Enns et al. (15) 2002 | Gehrmann (40) et al. 2003 | Aronica et al. (41) 2005 | Wurm et al. (42) 2007 | Wurm et al. (42) 2007 | Arnoux et al. (43) 2008 | Coman et al. (34) 2008 | Truin et al. (11) 2008 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient number | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 |
| Is hypoglycemia main presentation type? | No | Yes, | Yes, | No | No | No | No | No | No | Yes | No | No |
| Time of first attack of hypoglycemia | 5 wk | 1-3 mo | 3 mo | N/A | N/A | N/A | 1 mo | 1-2 wk | 1-2 wk | 2 mo | 1-3 wk | 0-1 yr |
| At hypoglycemia | | | | | | | | | | | | |
| Serum glucose (mg/dL) | 53.9 | 25.7 | N/A | 39.6 | N/A | N/A | 12.8 | N/A | N/A | 48.6 | 9 | N/A |
| Serum insulin (µIU/mL) | N/A | 4.8 | N/A | 2.5 | N/A | N/A | N/A | N/A | N/A | 1.2 | 4.0 | N/A |
| Serum cortisol (µg/dL) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Serum growth hormone (ng/dL) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Ketones | N/A | (-) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Reason for hypoglycemia | N/A | HI | N/A | HI | N/A | N/A | N/A | N/A | N/A | HI | HI | N/A |
| Treatment | N/A | Dia, Panc. | N/A | Dia | N/A | N/A | N/A | N/A | N/A | Dia | Dia | N/A |
| Duration of hypoglycemia (permanent/transient) | N/A | P | T | P | T | N/A | N/A | N/A | N/A | T | P | P |
| Duration of treatment | N/A | 2.5 y** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 24 mo | 3 wk | N/A |
| Outcome (death/alive) | Death | Alive | Alive | Alive | Alive | Death | Death | Death | Death | Alive | Death | Death |
| Age at death | 7 mo | | | | | 6 wk | 1 mo | 3 wk | 8 wk | | 3 wk | 6 y |
| Reason of death*** | A | | | | | B | C | D | D | | B | E |
| PMM2 molecular genetic testing**** | N/A | P113L / R141H | R141H / V231M | c.67-1G>A / L104V | F119L / R141H | N/A | Q55Afs*5 / T237R | Q55Afs*5 / V129L | Q55Afs*5 / V129L | C9Afs*27 / P113L | D148N / V231M | F119L / R141H |

*Low dose ACTH stimulation test: Normal.

** Diazoxide is discontinued due to side effect of diazoxide -hypotonic dehydration/hyponatremia.

***A.Massive hematemesis secondary to hepatocellular insufficiency B.Cardiac Tamponade C.Circulatory and respiratory failure D.Acute cardiac failure E.Acute episode of ascites and pericardial effusion production F. Epilepsia partialis continua

****NCBI reference sequences: NC_000016.10, NM_000303.3, NP_000294.1.

M: male, F: female, HI: hyperinsulinism, Dia: diazoxide HCTZ: hydrochlorothiazide, Panc: pancreatectomy (near-total), T: transient, P: permanent, N/A: not available

| Truin et al. (11) 2008 | Truin et al. (11) 2008 | Malhotra et al. (35) 2009 | Shanti et al. (19) 2009 | Shanti et al. (19) 2009 | Shanti et al. (19) 2009 | Rudaks et al. (44) 2012 | Serrano et al. (45) 2015 | Al Teneiji et al. (46) 2017 | Kasapkara et al. (36) 2017 | Vurallı 2021 (Case 1) | Vurallı 2021 (Case 2) | Vurallı 2021 (Case 3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P13 | P14 | P15 | P16 | P17 | P18 | P19 | P20 | P21 | P22 | P23 | P24 | P25 |
| No | No | No | Yes | No | Yes | Yes | No | No | No | No | No | No |
| 1-4 wk | 0-1 yr | 1-4 wk | 9 d | 8 mo | 1-4 wk | 5 d | N/A | N/A | N/A | 6 mo | 8 mo | 1-4 wk |
| N/A | N/A | N/A | 40.3 | 47.7 | 42.2 | 40.3 | N/A | 31.2 | N/A | 46 | 36 | 38 |
| N/A | N/A | High | 4.8 | 3.3 | 3.4 | N/A | N/A | 1.7 | High | 6 | 3.1 | 1.7 |
| N/A | N/A | N/A | N/A | Normal | N/A | N/A | N/A | N/A | N/A | 35 | 6.19* | 11.5* |
| N/A | N/A | N/A | N/A | Normal | N/A | N/A | N/A | N/A | N/A | 19.6 | 8.01 | 38 |
| N/A | N/A | N/A | N/A | N/A | (-) | N/A | N/A | N/A | N/A | (-) | (-) | (-) |
| N/A | N/A | HI | HI | HI | HI | N/A | N/A | HI | HI | HI | HI | HI |
| N/A | N/A | N/A | Dia | Dia, HCTZ | Dia | N/A | N/A | Dia | Dia | Not initiated | Dia | Dia |
| N/A | P | N/A | P | T | P | N/A | N/A | T | P | P | T | Pt |
| N/A | N/A | N/A | N/A | 7 yr | N/A | N/A | N/A | 15 mo | N/A | N/A | 6 mo | N/A |
| Alive | Death | Death | Alive | Alive | Death | Death | Alive | Alive | Alive | Death | Alive | Alive |
| | 1.5 y<br>E | 3.5 wks<br>B | | | 14 yr | 12 d<br>B | | | | 6 mo<br>F | | |
| c.640-15479C>T / V231M | L35* / D118G | D148N / V231M | I132T / F207S | F157S / unknown | R141H / V231M | R141H / V231M | T118I / P184T | G208A/ R123Q | V129M / V129M | R141H / V231M | V129M / V129M | I120T / G117C |

were low in three cases, TSH was high in six cases and only T4 was low in one case. While three of the cases with high TSH also had accompanying low T4 (clinical hypothyroidism), T4 values were not given in the remaining three. One of the hypoglycemic cases also had hypocholesterolemia and another case had hypertriglyceridemia.

There was no difference in terms of demographic data, such as age at presentation and diagnosis, gender and accompanying organ involvement between hyperinsulinemic cases and cases with normal or unspecified insulin level (Supplementary Table 1). Nine of ten patients with hyperinsulinism were started on oral diazoxide treatment and all but one patient responded to diazoxide (Figure 1). The patient who did not respond to diazoxide had persistent hyponatremia as a side effect of diazoxide and diazoxide could not be continued. Glucose levels returned to normal in this case only after subtotal pancreatectomy (32). Treatment approach was not reported in one case with hyperinsulinemic hypoglycemia. Of the patients who needed diazoxide treatment to control hypoglycemia, hypoglycemia was transient in three patients and permanent in the others (Figure 1). One of the patients with transient hypoglycemia discontinued treatment after using diazoxide for 15 months, another for 24 months and another for 7 years. All cases with transient hyperinsulinism were alive at the time of reporting. One of the patients with permanent hyperinsulinemic hypoglycemia died at the age of 3 weeks, another at 3.5 weeks and another at the age of 14 years (19,34). There was severe biventricular hypertrophic cardiomyopathy and pericardial effusion in the prenatal period in cases who died at a very early age of 3 and 3.5 weeks, and the cause of death in both patients was severe pericardial tamponade (34,35). The cause of death in the child, who died at the age of 14, was not specified. Data on the etiology of hypoglycemia, treatment methods and outcome were lacking in patients who were not reported to be hyperinsulinemic. While two thirds (8/12) of the cases with undefined etiology of hypoglycemia died due to various causes, usually during the infancy period, this ratio was one third (4/13) in cases with hyperinsulinism (Figure 1).

Genotype analysis of the patients with hypoglycemia did not reveal any correlation to the phenotype with respect to hyperinsulinism. Missense mutations have been reported in affected individuals, causing amino acid alterations in conserved residues. Most cases with hypoglycemia were compound heterozygous. The most common mutant alleles in the hypoglycemic patients were p.Arg141His and p.Val231Met; and their compound heterozygosity was the most common genotype, associated with severe disease and mortality similar to the general PMM2-CDG population. Other relatively common mutant alleles were p.Phe119Leu, p.Val129Met, and p.Pro113Leu (Table 1). Homozygous known pathogenic variants p.Val129Met/p.Val129Met were present in two Turkish cases, including one reported in this paper. As neither of these patients had consanguineous parents, increased allele frequency of this variant in the Turkish population has been proposed, but not demonstrated (27,36). One of the cases with this genotype had transient and the other had permanent hyperinsulinism (Patient 22 and 24).

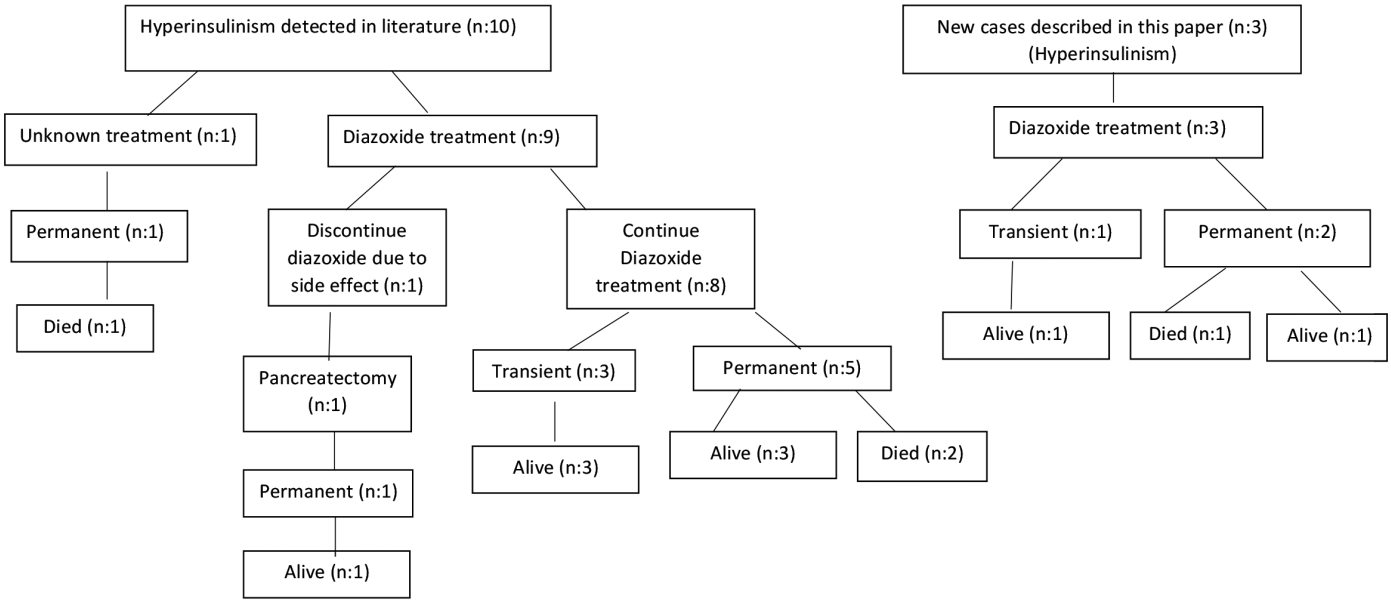


**Figure 1.** Etiology and outcome of hypoglycemic PMM2-CDG patients

*PMM2-CDG: Phosphomannomutase 2 deficiency*

## Discussion

The literature review indicated that hypoglycemia is a rare finding seen in PMM2-CDG. Including the three new cases described in this article, hypoglycemia was detected in only 25 of 1064 patients in total. In about one fourth of the PMM2-CDG cases with hypoglycemia, hypoglycemia was the main presenting finding and symptoms, such as seizures and decreased consciousness associated with hypoglycemia, have been reported in these cases. However, in the remainder, no major symptom or sign related to hypoglycemia was observed, and hypoglycemia was discovered during routine investigations. This situation suggests that some cases with hypoglycemia may remain undetected since there are no symptoms specific to hypoglycemia, especially in cases with severe neurological deficits. Therefore, we suggest close monitoring of blood glucose in cases with CDG, even if there is no clinical finding related to hypoglycemia.

Half of the cases with hypoglycemia had documented hyperinsulinism. However, in the other half, the cause of hypoglycemia could not be determined, since there is not sufficient data. Hormone levels, including cortisol and GH whose deficiencies could cause hypoglycemia, and other laboratory investigations, such as serum or urine ketone levels, were not reported in most cases. A complete etiological evaluation is essential to determine the cause of hypoglycemia and to plan the appropriate treatment. Although it is difficult to interpret the etiology due to the lack of data, hyperinsulinemic hypoglycemia may be the main cause of hypoglycemia in cases with PMM2-CDG. Besides, no case of hypoglycemia due to cortisol or GH deficiency is reported in the literature in patients with PMM2-CDG. Probably because the cases have not been investigated in detail in this respect, hyperinsulinemic hypoglycemia has been rarely reported in cases with PMM2-CDG. It is possible that at least some of the cases of hypoglycemia of unknown cause may be attributable to hyperinsulinism.

Cases with PMM2-CDG should be evaluated for possible hypoglycemia, and if it is documented further analysis for etiology, especially hyperinsulinism, should be carried out. We believe that if a detailed etiological evaluation of each hypoglycemia case is made properly, the majority of PMM2-CDG cases will have hyperinsulinism as the etiology and, with the appropriate treatment, hypoglycemia, which has a high probability of severe sequelae if left untreated, can be prevented.

One of the cases in the current series (case 3) did not have a high level of insulin at the time of hypoglycemia although it was measurable, but this patient responded well to diazoxide. Therefore, patients with PMM2-CDG can benefit from diazoxide treatment even if insulin levels were not so high. It should also be noted that poor oral feeding, feeding intolerance, vomiting and diarrhea, and hepatic dysfunction may contribute to hypoglycemia. Optimization of feeding may contribute to better glycemic control, as witnessed in cases 2 and 3.

Hypoglycemia seems to appear during early infancy in cases with PMM2-CDG but the age of diagnosis may be delayed. Failure to thrive is a common finding (approximately 75%) in cases with hypoglycemia. All organ system involvement, but especially central nervous system and gastrointestinal system involvement, can be seen in these cases with variable frequencies. Besides, CDG specific findings, such as inverted nipples and abnormal fat distribution, liver, kidney, and cardiac involvement are observed in 2/3 of the cases with hypoglycemia, and coagulopathies are observed in approximately half of the cases. More rarely, skeletal findings, thrombocytopenia, fetal hydrops, acute pancreatitis, stroke-like episodes, peripheral neuropathy, and athetosis have been described in these cases. Hyperinsulinemic hypoglycemia is usually a part of a multisystemic disease with different organ involvement. The only exception is a case who had no serious organ involvement other than hyperinsulinism (19). While this case was followed up due to failure to thrive, she had afebrile seizures due to hypoglycemia at the age of 8 months and hyperinsulinism was determined as the etiology of her hypoglycemia. She did not have signs more specific to CDG, such as strabismus, inverted nipples or an abnormal fat pad distribution. Her hypoglycemia responded well to diazoxide and she was being followed up as a case with normal psychomotor development and no cerebellar abnormality. Since the age of this case was young when published, it is also possible that other organ involvements might become more pronounced during follow-up.

There was no difference in terms of demographic and clinical findings between cases with hyperinsulinism and cases with unspecified insulin level. In hyperinsulinemic cases, no pancreatic pathology was detected to explain hyperinsulinism. All cases in whom oral diazoxide treatment was initiated responded well to this treatment. In only one case, diazoxide treatment was discontinued due to severe hyponatraemia with fluid retention, which is a side effect of diazoxide. It was not stated whether other medical treatment options were evaluated in that case, but it was reported that he had subtotal pancreatectomy due to hyperinsulinism. Including the three new patients presented in this article, hyperinsulinism was permanent in two thirds (9/13) of the cases, while it was transient in the remaining one third (4/13). In cases where hyperinsulinism was transient, diazoxide treatment was usually discontinued one or two

years after the initiation of treatment. In one case, it was discontinued after using diazoxide for a longer period of 7 years. Half of the patients with permanent, patients died mostly due to cardiac abnormalities, while no patient with transient hyperinsulinism died. No genotype-phenotype correlation was observed in the cases with respect to hyperinsulinism. It is interesting to note that while the most common p.Arg141His allele is reported to be present in approximately 60% of reported cases of PMM2-CDG (30), this allele is slightly underrepresented (30%) in those with hypoglycemia (7 of 23 patients with known genotypes; Table 1).

It is unclear why hyperinsulinism is seen in cases with PMM2-CDG, and the pathophysiology is not fully explained. Hypoglycosylation of sulfonylurea receptor 1 (SUR1) and other proteins involved in insulin secretion has been suggested as the responsible mechanism. It is known that all PMM2-CDG patients with hyperinsulinemic hypoglycemia respond well to diazoxide. Diazoxide directly acts on beta cells in the pancreas, opening the KATP channel and preventing insulin release from beta cells (37). The good response of the cases with PMM2-CDG to diazoxide suggests that the hyperinsulinism seen in these cases may be due to the impaired function of KATP channels. KATP channels contain four ion channels (Kir6.2) and four regulatory SUR1 receptors and control glucose-stimulated release of insulin. Glycosylation of SUR1 is required for the expression of KATP channels on the surface (38). In addition, the dysfunction of the insulin receptor may be the cause of the hyperinsulinism seen in these cases. The insulin receptor consists of two extracellular $\alpha$ subunits and two transmembrane $\beta$ subunits, and both subunits are glycosylated. Each of the $\alpha$ monomers contains 13 N-glycans, while the $\beta$-monomers contain four N-glycans and six O-glycans. It is not known whether glycosylation of insulin receptors is affected in CDGs (39). The insulin level in one case described in this article was not markedly elevated at the time of hypoglycemia. A possible hypothesis for this case may be hypoglycolization at the postreceptor level, and even if insulin levels are not very high, hyperinsulinism findings and hypoketotic hypoglycemia may be observed in these cases, similar to *AKT2* activating mutation.

### Study Limitations

Although hypoglycemia was detected in 37 cases with PMM2-CDG during the literature review, only 22 cases could be evaluated in detail due to the lack of sufficient clinical and laboratory data.

## Conclusion

The main cause of hypoglycemia in PMM2-CDG appears to be hyperinsulinism. Abnormal counter-regulatory hormone response has not been identified in cases with PMM2-CDG, suggesting hyperinsulinism may still be the underlying cause of hypoglycemia in those with undetermined cause. Other possible causes of hypoglycemia, such as inadequate feeding, malnutrition, chronic diarrhea, and hepatic disease may also contribute to low levels of blood glucose. In cases with PMM2-CDG, even if there are no symptoms specific to hypoglycemia, blood glucose should be closely monitored and a detailed etiological evaluation should be performed when hypoglycemia is detected. Although insulin levels taken at the time of hypoglycemia may not be very high, hypoglycemia seen in patients with PMM2-CDG may respond well to diazoxide treatment.

### Ethics

**Ethics Committee Approval:** This study protocol was reviewed and approved by Hacettepe University Ethics Committee (approval number GO 17/141-14).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Doğuş Vurallı, Yılmaz Yıldız, Alev Ozon, Ali Dursun, Nazlı Gönç, Ayşegül Tokatlı, H. Serap Sivri, Ayfer Alikaşifoğlu, Concept: Doğuş Vurallı, Yılmaz Yıldız, H. Serap Sivri, Design: Doğuş Vurallı, Yılmaz Yıldız, H. Serap Sivri, Data Collection or Processing: Doğuş Vurallı, Alev Ozon, Ayşegül Tokatlı, Analysis or Interpretation: Doğuş Vurallı, Yılmaz Yıldız, Alev Ozon, Ali Dursun, Nazlı Gönç, Ayşegül Tokatlı, H. Serap Sivri, Ayfer Alikaşifoğlu, Literature Search: Doğuş Vurallı, Alev Ozon, Writing: Doğuş Vurallı, Yılmaz Yıldız, Alev Ozon, H. Serap Sivri, Ayfer Alikaşifoğlu.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Péanne R, de Lonlay P, Foulquier F, Kornak U, Lefeber DJ, Morava E, Pérez B, Seta N, Thiel C, Van Schaftingen E, Matthijs G, Jaeken J. Congenital disorders of glycosylation (CDG): Quo vadis? Eur J Med Genet 2018;61:643-663. Epub 2017 Oct 25
2. de Lonlay P, Seta N, Barrot S, Chabrol B, Drouin V, Gabriel BM, Journel H, Kretz M, Laurent J, Le Merrer M, Leroy A, Pedespan D, Sarda P, Villeneuve N, Schmitz J, van Schaftingen E, Matthijs G, Jaeken J, Korner C, Munnich A, Saudubray JM, Cormier-Daire V. A broad spectrum of clinical presentations in congenital disorders of glycosylation I: a series of 26 cases. J Med Genet 2001;38:14-19.

3. Grünewald S, Imbach T, Huijben K, Rubio-Gozalbo ME, Verrips A, de Klerk JB, Stroink H, de Rijk-van Andel JF, Van Hove JL, Wendel U, Matthijs G, Hennet T, Jaeken J, Wevers RA. Clinical and biochemical characteristics of congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. Ann Neurol 2000;47:776-781.
4. Erlandson A, Bjursell C, Stibler H, Kristiansson B, Wahlström J, Martinsson T. Scandinavian CDG-Ia patients: genotype/phenotype correlation and geographic origin of founder mutations. Hum Genet 2001;108:359-367.
5. Monin ML, Mignot C, De Lonlay P, Héron B, Masurel A, Mathieu-Dramard M, Lenaerts C, Thauvin C, Gérard M, Roze E, Jacquette A, Charles P, de Baracé C, Drouin-Garraud V, Khau Van Kien P, Cormier-Daire V, Mayer M, Ogier H, Brice A, Seta N, Héron D. 29 French adult patients with PMM2-congenital disorder of glycosylation: outcome of the classical pediatric phenotype and depiction of a late-onset phenotype. Orphanet J Rare Dis 2014;9:207.
6. Thompson DA, Lyons RJ, Russell-Eggitt I, Liasis A, Jagle H, Grünewald S. Retinal characteristics of the congenital disorder of glycosylation PMM2-CDG. J Inherit Metab Dis 2013;36:1039-1047. Epub 2013 Feb 22
7. Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. Eur J Pediatr 2003;162:359-379. Epub 2003 Mar 15
8. Kristiansson B, Stibler H, Hagberg B, Wahlström J. [CDGS-1--a recently discovered hereditary metabolic disease. Multiple organ manifestations, incidence 1/80,000, difficult to treat]. Lakartidningen 1998;95:5742-5748.
9. Marquardt T, Hülskamp G, Gehrmann J, Debus V, Harms E, Kehl HG. Severe transient myocardial ischaemia caused by hypertrophic cardiomyopathy in a patient with congenital disorder of glycosylation type Ia. Eur J Pediatr 2002;161:524-527. Epub 2002 Aug 22
10. Romano S, Bajolle F, Valayannopoulos V, Lyonnet S, Colomb V, de Baracé C, Vouhe P, Pouard P, Vuillaumier-Barrot S, Dupré T, de Keyzer Y, Sidi D, Seta N, Bonnet D, de Lonlay P. Conotruncal heart defects in three patients with congenital disorder of glycosylation type Ia (CDG Ia). J Med Genet 2009;46:287-288.
11. Truin G, Guillard M, Lefeber DJ, Sykut-Cegielska J, Adamowicz M, Hoppenreijs E, Sengers RCA, Wevers RA, Morava E. Pericardial and abdominal fluid accumulation in congenital disorder of glycosylation type Ia. Mol Genet Metab 2008;94:481-484. Epub 2008 Jun 20
12. Barone R, Carrozzi M, Parini R, Battini R, Martinelli D, Elia M, Spada M, Lilliu F, Ciana G, Burlina A, Leuzzi V, Leoni M, Sturiale L, Matthijs G, Jaeken J, Di Rocco M, Garozzo D, Fiumara A. A nationwide survey of PMM2-CDG in Italy: high frequency of a mild neurological variant associated with the L32R mutation. J Neurol 2015;262:154-164. Epub 2014 Oct 30
13. Krasnewich D, O'Brien K, Sparks S. Clinical features in adults with congenital disorders of glycosylation type Ia (CDG-Ia). Am J Med Genet C Semin Med Genet 2007;145:302-306.
14. Krasnewich D, Gahl WA. Carbohydrate-deficient glycoprotein syndrome. Adv Pediatr 1997;44:109-140.
15. Enns GM, Steiner RD, Buist N, Cowan C, Leppig KA, McCracken MF, Westphal V, Freeze HH, O'brien JF, Jaeken J, Matthijs G, Behera S, Hudgins L. Clinical and molecular features of congenital disorder of glycosylation in patients with type 1 sialotransferrin pattern and diverse ethnic origins. J Pediatr 2002;141:695-700.
16. Pérez Jde J, Udeshi ND, Shabanowitz J, Ciordia S, Juárez S, Scott CL, Olszewski NE, Hunt DF, García JA. O-GlcNAc modification of the coat protein of the potyvirus Plum pox virus enhances viral infection. Virology 2013;442:122-131. Epub 2013 Apr 29
17. Kjaergaard S, Schwartz M, Skovby F. Congenital disorder of glycosylation type Ia (CDG-Ia): phenotypic spectrum of the R141H/F119L genotype. Arch Dis Child 2001;85:236-239.
18. Miller BS, Freeze HH. New disorders in carbohydrate metabolism: congenital disorders of glycosylation and their impact on the endocrine system. Rev Endocr Metab Disord 2003;4:103-113.
19. Shanti B, Silink M, Bhattacharya K, Howard NJ, Carpenter K, Fietz M, Clayton P, Christodoulou J. Congenital disorder of glycosylation type Ia: heterogeneity in the clinical presentation from multivisceral failure to hyperinsulinaemic hypoglycaemia as leading symptoms in three infants with phosphomannomutase deficiency. J Inherit Metab Dis 2009;32(Suppl 1):241-251. Epub 2009 Apr 27
20. de Zegher F, Jaeken J. Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. Pediatr Res 1995;37:395-401.
21. Kristiansson B, Stibler H, Wide L. Gonadal function and glycoprotein hormones in the carbohydrate-deficient glycoprotein (CDG) syndrome. Acta Paediatr 1995;84:655-659.
22. Mohamed M, Theodore M, Claahsen-van der Grinten H, van Herwaarden AE, Huijben K, van Dongen L, Kouwenberg D, Lefeber DJ, Wevers RA, Morava E. Thyroid function in PMM2-CDG: diagnostic approach and proposed management. Mol Genet Metab 2012;105:681-683. Epub 2012 Feb 13
23. Kranz C, Basinger AA, Güçsavaş-Calikoğlu M, Sun L, Powell CM, Henderson FW, Aylsworth AS, Freeze HH. Expanding spectrum of congenital disorder of glycosylation Ig (CDG-Ig): sibs with a unique skeletal dysplasia, hypogammaglobulinemia, cardiomyopathy, genital malformations, and early lethality. Am J Med Genet A 2007;143:1371-1378.
24. Miller BS, Freeze HH, Hoffmann GF, Sarafoglou K. Pubertal development in ALG6 deficiency (congenital disorder of glycosylation type Ic). Mol Genet Metab 2011;103:101-103. Epub 2011 Feb 3
25. Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. Congenital disorder of glycosylation id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. J Clin Endocrinol Metab 2005;90:4371-4375. Epub 2005 Apr 19
26. Moravej H, Altassan R, Jaeken J, Enns GM, Ellaway C, Balasubramaniam S, De Lonlay P, Coman D, Mercimek-Andrews S, Witters P, Morava E. Hypoglycemia in CDG patients due to PMM2 mutations: Follow up on hyperinsulinemic patients. JIMD Rep 2020;51:76-81.
27. Yıldız Y, Arslan M, Çelik G, Kasapkara ÇS, Ceylaner S, Dursun A, Sivri HS, Coşkun T, Tokatlı A. Genotypes and estimated prevalence of phosphomannomutase 2 deficiency in Turkey differ significantly from those in Europe. Am J Med Genet A 2020;182:705-712. Epub 2020 Jan 25
28. Matthijs G, Schollen E, Bjursell C, Erlandson A, Freeze H, Imtiaz F, Kjaergaard S, Martinsson T, Schwartz M, Seta N, Vuillaumier-Barrot S, Westphal V, Winchester B. Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). Hum Mutat 2000;16:386-394.
29. 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
30. Altassan R, Péanne R, Jaeken J, Barone R, Bidet M, Borgel D, Brasil S, Cassiman D, Cechova A, Coman D, Corral J, Correia J, de la Morena-Barrio ME, de Lonlay P, Dos Reis V, Ferreira CR, Fiumara A, Francisco R, Freeze H, Funke S, Gardeitchik T, Gert M, Girad M, Giros M, Grünewald

S, Hernández-Caselles T, Honzik T, Hutter M, Krasnewich D, Lam C, Lee J, Lefeber D, Marques-de-Silva D, Martinez AF, Moravej H, Ōunap K, Pascoal C, Pascreau T, Patterson M, Quelhas D, Raymond K, Sarkhail P, Schiff M, Seroczyńska M, Serrano M, Seta N, Sykut-Cegielska J, Thiel C, Tort F, Vals MA, Videira P, Witters P, Zeevaert R, Morava E. International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: Diagnosis, treatment and follow up. J Inherit Metab Dis 2019;42:5-28.

31. Witters P, Honzik T, Bauchart E, Altassan R, Pascreau T, Bruneel A, Vuillaumier S, Seta N, Borgel D, Matthijs G, Jaeken J, Meersseman W, Cassiman D, Pascale de L, Morava E. Long-term follow-up in PMM2-CDG: are we ready to start treatment trials? Genet Med 2019;21:1181-1188. Epub 2018 Oct 8
32. Böhles H, Sewell AA, Gebhardt B, Reinecke-Lüthge A, Klöppel G, Marquardt T. Hyperinsulinaemic hypoglycaemia--leading symptom in a patient with congenital disorder of glycosylation Ia (phosphomannomutase deficiency). J Inherit Metab Dis 2001;24:858-862.
33. Antoun H, Villeneuve N, Gelot A, Panisset S, Adamsbaum C. Cerebellar atrophy: an important feature of carbohydrate deficient glycoprotein syndrome type 1. Pediatr Radiol 1999;29:194-198.
34. Coman D, Bostock D, Hunter M, Kannu P, Irving M, Mayne V, Fietz M, Jaeken J, Savarirayan R. Primary skeletal dysplasia as a major manifesting feature in an infant with congenital disorder of glycosylation type Ia. Am J Med Genet A 2008;146:389-392.
35. Malhotra A, Pateman A, Chalmers R, Coman D, Menahem S. Prenatal cardiac ultrasound finding in congenital disorder of glycosylation type 1a. Fetal Diagn Ther 2009;25:54-57. Epub 2009 Jan 29
36. Kasapkara ÇS, Barış Z, Kılıç M, Yüksel D, Keldermans L, Matthijs G, Jaeken J. PMM2-CDG and sensorineural hearing loss. J Inherit Metab Dis 2017;40:629-630. Epub 2017 Jul 31
37. Virgili N, Mancera P, Wappenhans B, Sorrosal G, Biber K, Pugliese M, Espinosa-Parrilla JF. K(ATP) channel opener diazoxide prevents neurodegeneration: a new mechanism of action via antioxidative pathway activation. PLoS One 2013;8:e75189.
38. Conti LR, Radeke CM, Vandenberg CA. Membrane targeting of ATP-sensitive potassium channel. Effects of glycosylation on surface expression. J Biol Chem 2002;277:25416-25422. Epub 2002 May 6
39. Belfiore A, Malaguarnera R, Vella V, Lawrence MC, Sciacca L, Frasca F, Morrione A, Vigneri R. Insulin Receptor Isoforms in Physiology and Disease: An Updated View. Endocr Rev 2017;38:379-431.
40. Gehrmann J, Sohlbach K, Linnebank M, Böhles HJ, Buderus S, Kehl HG, Vogt J, Harms E, Marquardt T. Cardiomyopathy in congenital disorders of glycosylation. Cardiol Young 2003;13:345-351.
41. Aronica E, van Kempen AA, van der Heide M, Poll-The BT, van Slooten HJ, Troost D, Rozemuller-Kwakkel JM. Congenital disorder of glycosylation type Ia: a clinicopathological report of a newborn infant with cerebellar pathology. Acta Neuropathol 2005;109:433-442. Epub 2005 Feb 1
42. Wurm D, Hänsgen A, Kim YJ, Lindinger A, Baghai A, Gortner L. Early fatal course in siblings with CDG-Ia (caused by two novel mutations in the PMM2 gene): clinical, molecular and autopsy findings. Eur J Pediatr 2007;166:377-378. Epub 2006 Aug 29
43. Arnoux JB, Boddaert N, Valayannopoulos V, Romano S, Bahi-Buisson N, Desguerre I, de Keyzer Y, Munnich A, Brunelle F, Seta N, Dautzenberg MD, de Lonlay P. Risk assessment of acute vascular events in congenital disorder of glycosylation type Ia. Mol Genet Metab 2008;93:444-449. Epub 2008 Feb 21
44. Rudaks LI, Andersen C, Khong TY, Kelly A, Fietz M, Barnett CP. Hypertrophic cardiomyopathy with cardiac rupture and tamponade caused by congenital disorder of glycosylation type Ia. Pediatr Cardiol 2012;33:827-830. Epub 2012 Feb 29
45. Serrano M, de Diego V, Muchart J, Cuadras D, Felipe A, Macaya A, Velázquez R, Poo MP, Fons C, O'Callaghan MM, García-Cazorla A, Boix C, Robles B, Carratalá F, Girós M, Briones P, Gort L, Artuch R, Pérez-Cerdá C, Jaeken J, Pérez B, Pérez-Dueñas B. Phosphomannomutase deficiency (PMM2-CDG): ataxia and cerebellar assessment. Orphanet J Rare Dis 2015;10:138.
46. Al Teneiji A, Bruun TU, Sidky S, Cordeiro D, Cohn RD, Mendoza-Londono R, Moharir M, Raiman J, Siriwardena K, Kyriakopoulou L, Mercimek-Mahmutoglu S. Phenotypic and genotypic spectrum of congenital disorders of glycosylation type I and type II. Mol Genet Metab 2017;120:235-242. Epub 2017 Jan 3

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2022-12-4

J Clin Res Pediatr Endocrinol 2022;14(3):287-292

# Incidence of Newly Diagnosed Type 1 Diabetes Mellitus in Children and Adolescents in Henan Province of China from 2017 to 2020: A Retrospective Multicenter Study Based on Hospitalization Data

**Qiong Chen[1], Na Xu[2], Yongxing Chen[1], Mingming Yan[3], Fengyan Tian[4], Wei Yang[1], Yan Cui[1], Ai Huang[1], Yangshiyu Li[1], He Zhang[5], Zhihong Jiang[6], Ruizhi Zheng[7], Yuan Ji[8], Dongming Zhang[9], Qiao Ren[10], Li Ding[11], Haiyan Wei[1]**

*[1]Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Department of Endocrinology, Genetics and Metabolism, Henan, China*
*[2]Nanyang Central Hospital, Department of Endocrinology, Henan, China*
*[3]Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Henan Key Laboratory of Children's Genetics and Metabolic Diseases, Henan, China*
*[4]The First Affiliated Hospital of Zhengzhou University, Department of Pediatrics, Henan, China*
*[5]The First Affiliated Hospital of Xinxiang Medical University, Department of Endocrinology, Henan, China*
*[6]The First Affiliated Hospital of Henan University of Science and Technology, Department of Endocrinology, Henan, China*
*[7]Henan Provincial People's Hospital, Department of Endocrinology, Henan, China*
*[8]Kaifeng Children's Hospital, Department of Endocrinology, Henan, China*
*[9]The Second Affiliated Hospital of Zhengzhou University, Department of Endocrinology, Henan, China*
*[10]989 Hospital of PLA Joint Logistics Support Force, Department of Endocrinology, Henan, China*
*[11]Anyang Maternal and Childcare Hospital, Department of Pediatrics, China*

### What is already known on this topic?

The incidence of type 1 diabetes mellitus (T1DM) is rapidly increasing worldwide. A national study including 13 cities reported that the incidence per 100,000 person years in China was 1.93 (0.83-3.03) for the 0-14 years age group from 2010 to 2013. The incidence of T1DM varied between regions.

### What this study adds?

Henan province was defined as a less developed region in China. However, the incidence in Henan Province of China has been unknown for more than two decades. Our study investigated the incidence of newly diagnosed T1DM cases between 2017 and 2020 in Henan province of China.

## Abstract

**Objective:** The incidence of type 1 diabetes mellitus (T1DM) is rapidly increasing worldwide. However, the incidence in Henan Province of China has been unknown for more than two decades. This study aimed to estimate the incidence of T1DM in the 0.5-14.9 years age group in Henan Province of China from 2017 to 2020.

**Methods:** A retrospective analysis of hospital registration data from 18 cities in Henan Province, China, identified 1726 patients (843 males, 883 females) between 0.5-14.9 years of age with newly diagnosed T1DM in Henan Province from January 1st, 2017, to December 31st, 2020, covering more than 19 million children years at risk.

**Address for Correspondence:** Haiyan Wei MD, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Department of Endocrinology, Genetics and Metabolism, Henan, China
**Phone:** +186-13838501138 **E-mail:** haiyanwei2009@163.com **ORCID:** orcid.org/0000-0003-1044-6594

**Conflict of interest:** None declared
**Received:** 19.01.2022
**Accepted:** 25.03.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

J Clin Res Pediatr Endocrinol
2022;14(3):287-292

**Results:** The crude incidence of T1DM per 100 000 person years for the 0.5-14.9 years age group in the Henan Province of China was 2.19 [95% confidence interval (CI): 1.99, 2.40], with a peak in the 10-14.9 years age group. The rate ratio of females to males was 1.32 (95% CI: 1.20, 1.45) in the 0.5-14.9 years age group. The incidence rate was higher in females than males in the 5-9.9 years age group ($p < 0.01$) and the 10-14.9 years age group ($p < 0.01$). The seasonality of the incidence was different from that in previous reports, with the lowest incidence in the spring.

**Conclusion:** The incidence of T1DM in the 0.5-14.9 years age group in Henan Province was still among the lowest reported globally, but was in line with other incidence rates reported from China.

**Keywords:** Incidence, type 1 diabetes mellitus, children, China, new onset

## Introduction

The incidence of type 1 diabetes mellitus (T1DM) in children has been rapidly increasing worldwide. According to the DiaMond (Diabetes Mondiale) Project, the incidence of T1DM in Chinese children was 0.51 per 100,000 person years from 1988 to 1996, which was one of the lowest in the world (1). A national study including 13 cities reported that the incidence per 100,000 person years in China was 1.93 (0.83-3.03) for the 0-14 years age group from 2010 to 2013 (2). However, the population of the national study did not include residents of Henan Province. Henan Province is in the center of China, located at latitude 31°23-36°22 North and longitude 110°21-116°39 East, with an average altitude of 100 m above sea level.

Based on the gross domestic product for each province, Henan was defined as a less developed region in China. The DiaMond Project reported that from 1989 to 1994, the incidence of T1DM in 0-14 year old children was 0.5/100,000 in Zhengzhou, the capital city of Henan Province (1). The incidence of T1DM in Henan Province has thus been unknown for more than two decades. There is no nationwide registry for T1DM in Henan province of China, and both patients and their parents are reluctant to publicly disclose their disease because of traditional cultural values. Because of this, the capture-recapture method, which is thought to be the standard method of T1DM incidence survey, is not feasible in Henan Province. Instead, we chose a hospital data-based survey to estimate the incidence of T1DM, as in a previous study (3). In this study, we analyzed the incidence of childhood T1DM in Henan Province based on newly diagnosed and hospitalized T1DM cases from January 1st, 2017, to December 31st, 2020. This T1DM study may help advance the understanding of geographic and economic factors to the development of T1DM.

## Methods

The study design was approved by the ethics review board of Children's Hospital Affiliated to Zhengzhou University. We reviewed T1DM cases using hospital registration data from 137 hospitals located in 18 cities in Henan Province. Records were audited by senior pediatric endocrinologists in each hospital to rule out non-T1DM cases. Patients were eligible for study inclusion if they: 1) were 6 months to 14 years old; 2) were admitted to any of the selected hospitals from January 1st, 2017, to December 31st, 2020; and 3) were newly diagnosed with T1DM based on the 2009 International Society for Pediatric and Adolescent Diabetes guidelines and recommendations made by the World Health Organization Expert Committee. The age of T1DM onset was divided into three groups for consistency with previous studies (2): 0.5-4.9; 5-9.9; and 10-14.9 years. Data including sex, date of birth, race, and diabetes diagnosis date were collected from 1726 patients.

### Statistical Analysis

Crude incidence rates were calculated as the number of cases per 100,000 person-years, and the population of the sixth Chinese census conducted by the National Bureau of Statistics of China as the denominator. We estimated the incidence separately for three age groups at diagnosis (0.5-4.9, 5-9.9, and 10-14.9 years), and according to sex and season. The 95% confidence intervals (CIs) were calculated by inverting the score test for a binomial proportion. The incidence differences according to sex in the three age groups were evaluated using the $\chi^2$ test. Multivariate Poisson regression models were used to assess the effects of age group and sex on the incidence of diabetes. The incidence rate ratios (IRRs) and their 95% CIs were calculated from the regression coefficients and corresponding standard errors in the Poisson regression models. Statistical analyses were performed using the R4.0.5 analytical software (https://cran.r-project.org/). Statistical significance was set at $p < 0.05$.

## Results

Based on registration data from the selected hospitals, 1726 children (843 males, 883 females) with newly diagnosed T1DM were eligible for inclusion in the analysis. Incidence calculations for this patient population were derived from national census demographic statistics for 2010, in which 36.0%, 32.8%, and 31.2% of children in Henan Province

were 0.5-4.9 years, 5-9.9 years, and 10-14.9 years of age, respectively. The Han ethnicity accounts for 98.84% of the population in Henan province. In our study, 1714 children were from the Han ethnicity group, accounting for 99% of the total.

The incidence per 100,000 person years for the 0.5-14.9 years age group in the 18 cities of Henan province was 2.19 (95% CI: 1.99, 2.40) (Table 1). The incidence per 100,000 person years in the 0.5-4.9 years age group was 1.42 (95% CI: 1.16, 1.73), 1.39 (95% CI: 1.05, 1.81) among males, and 1.46 (95% CI: 1.07, 1.94) among females. The incidence per 100,000 person years in the 5-9.9 years age group was 1.82 (95% CI: 1.51, 2.18), 1.50 (95% CI: 1.13, 1.96) among males, and 1.82 (95% CI: 1.51, 2.18) among females. The incidence per 100,000 person years in the 10-14.9 years age group was 3.46 (95% CI: 3.01, 3.95), 2.96 (95% CI: 2.41, 3.59) among males, and 3.46 (95% CI: 3.01, 3.95) among females. Table 1 summarizes the incidence of T1DM in the different age groups and sexes.

The incidence rate was different between males and females. The female-to-male IRR was 1.32 (95% CI: 1.20, 1.45) in the 0.5-14.9 years age group. The incidence rates between males and females among different age groups are shown in Table 1. The incidence rate in females was higher than males in the 5-9.9 years age group ($p < 0.01$) and the 10-14.9 years age group ($p < 0.01$). There was no significant difference between males and females in the 0.5-4.9 years age group ($p = 0.5763$). The rate ratios were 1.29 (95% CI: 1.13-1.47) and 2.44 (95% CI: 2.16-2.74) in the 5-9.9 and 10-14.9 years age groups, respectively (Table 2).

People in Henan Province experience four distinct seasons a year: spring (March-May), summer (June-August), autumn (September-November), and winter (December-February). In Spring, the incidence rate was 1.92 (95% CI: 1.73, 2.12) per 100,000, lower than that in the other seasons (Table 3). The significant differences were found among spring, summer, and autumn ($p < 0.01$). The incidence rates between males and females in different seasons are also shown in Table 3.

## Discussion

The incidence of T1DM in the 0.5-14.9 years age group in Henan Province of China from 2017 to 2020, which has been unknown for more than two decades, was estimated in our study. Based on the data from 2019 International Diabetes Federation, T1DM incidence remains the highest in Finland (60/100,000/ year), and the lowest across East and South-East Asia (4). According to our study, the incidence rate in Henan province is one of the lowest compared to global rates, but similar to the overall incidence of China (2). China remains one of the countries with the lowest incidence of T1DM worldwide. The mechanism of low incidence in China is largely attributed to genetic, environmental, and behavioral factors. Our study population was Han people who have a low susceptibility to T1DM (1). Previous studies in China showed that the incidence of T1DM among children aged 0-14 years was strongly correlated with latitude (2). Moreover, less developed regions in China had lower prevalence compared to well developed regions (5). Henan Province is situated between 31°23 to 36°22 North, in the

**Table 1. Population and incidence of type 1 diabetes children aged 0.5-14.9 years in Henan Province of China**

| Age (years) | Number of cases | Population | Mean annual incidence rate (95% CI) per 100,000 |
|---|---|---|---|
| Males | | | |
| 0.5-4.9 | 55 | 3951617 | 1.39 (1.05, 1.81) |
| 5-9.9 | 55 | 3655027 | 1.50 (1.13, 1.96) |
| 10-14.9 | 101 | 3414352 | 2.96 (2.41, 3.59) |
| 0.5-14.9 | 211 | 11020996 | 1.91 (1.66, 2.19) |
| Females | | | |
| 0.5-4.9 | 46 | 3155452 | 1.46 (1.07, 1.94) |
| 5-9.9 | 63 | 2824983 | 2.23 (1.71, 2.85) |
| 10-14.9 | 112 | 2745887 | 4.08 (3.36, 4.91) |
| 0.5-14.9 | 221 | 8726322 | 2.53 (2.21, 2.89) |
| Total | | | |
| 0.5-4.9 | 101 | 7107069 | 1.42 (1.16, 1.73) |
| 5-9.9 | 118 | 6480010 | 1.82 (1.51, 2.18) |
| 10-14.9 | 213 | 6160239 | 3.46 (3.01, 3.95) |
| 0.5-14.9 | 432 | 19747318 | 2.19 ( 1.99, 2.40) |

CI: confidence interval

center of China, and is also considered a less developed region in China (Table 4) (2,6,7), which may partially explain these incidence levels.

The incidence of T1DM in the 0.5-14.9 years age group in 2017-2020 in our study suggests a 4.38-fold increase over that reported by the DiaMond study in Henan from 1989 to 1994, which is an approximately 6.63% annual increase. The annual increase is similar to that reported in previous studies in other cities of China (2). Compared with EURODIAB (3.3%) (8), the increase seems to be rapid. However, the reasons remain unclear. Westernized lifestyle (9), changes in the immune system (10), and vitamin D deficiency (11) may be associated with this increase. In addition, the increasing rate calculation is based on the data from the DiaMond study, which may be underestimated as some of the participating centers reported very small case numbers.

In addition, our study found that the incidence increased with age and the peak of incidence was within the 10-14.9 years age group, which is consistent with previous studies (12,13). The incidence of T1DM in children aged 10-14.9 years is 2.44 times as high as that in children aged 0.5-4.9 years. This high level of incidence may be related to the higher level of growth hormone, which may reduce insulin sensitivity and contribute to insulin deficiency (14). In our study, the incidence of T1DM in 0.5-4.9 age group was 1.47 per 100,000 from 2017 to 2020. Previous studies have shown that the incidence of diabetes in children aged 0-5 years increased significantly, reaching 5-35% in different studies in China (6,7). However, the rate of increase was not compared in this study.

Previous studies suggested that the incidence in males exceeds that in females in most countries where incidence is high (populations of European origin), but lower than that in females in low incidence countries (Asia and Africa) (2,15,16). In China, studies in Zhejiang, Harbin, and Hong Kong also reported a higher incidence in females than in males (6,17,18). In our study, the rate ratio of females was 1.32 (95% CI: 1.20, 1.93) in the 0-14 years age group. The higher incidence of females than males in Henan Province was consistent with previous studies.

Among the four seasons, newly diagnosed T1DM has long been described more commonly in winter, early spring, and late autumn months in Western countries (19). In a Japanese study, the peak periods of disease onset were spring and winter, which is similar to Hong Kong and southern China (16,18). However, a lack of seasonality was observed in a study from Shanghai (20). In our study, the incidence was lowest in spring, which was significantly different from that reported in previous studies. This difference may be due to many factors. A multicenter study in China showed that 4.17% cases of T1DM were diagnosed during routine physical examinations, and 11.44% of cases were diagnosed when seeking medical attention for illness symptoms, such as fever and cough (21). This suggests a lack of recognition for T1DM in public and this will lead to a delayed diagnosis and may also accout for the lower incidence in China. The different degrees of delayed diagnosis may result in inaccurate statistics on the onset date of diabetes. Moreover, social stigma in China may lower the number of children presenting to hospital or change the seasonality (22). Besides, environmental factors, such as viral infections, may also affect the seasonal variation. During the COVID-19 pandemic in China, infectious diseases such as influenza, bronchiolitis, and acute upper respiratory infections were obviously lower than previously reported (23). Changes in the disease spectrum in China may also change the seasonality of T1DM.

**Table 2. Incidence rate ratios and 95% CI of children in Henan Province of China**

| | RR | 95% CI |
|---|---|---|
| Male | 1 | |
| Female | 1.32 | 1.20, 1.45 |
| Age (year) | | |
| 0.5-4.9 | 1 | |
| 5-9.9 | 1.29 | 1.13, 1.47 |
| 10-14.9 | 2.44 | 2.16, 2.74 |

CI: confidence interval

**Table 3. Incidence rate of type 1 diabetes children aged 0.5-14.9 years among different seasons in Henan Province of China**

| Season | Incidence rate (95% CI) per 100,000 |
|---|---|
| Spring | 1.92 (1.73, 2.12) |
| Males | 1.68 (1.45, 1.94) |
| Females | 2.22 (1.92, 2.56) |
| Summer | 2.39 (2.17, 2.61) |
| Males | 2.11 (1.85, 2.40) |
| Females | 2.73 (2.39, 3.10) |
| Autumn | 2.35 (2.15, 2.58) |
| Males | 2.15 (1.89, 2.44) |
| Females | 2.61 (2.28, 2.97) |
| Winter | 2.08 (1.88, 2.29) |
| Males | 1.71 (1.47, 1.97) |
| Females | 2.56 (2.23, 2.91) |

CI: confidence interval

**Table 4. Crude incidence rates per 100 000 person years in various regions in mainland of China**

| Regions | Years | Age (y) | Crude incidence per 100 000 person years | Latitude (°N) | GDP status |
|---|---|---|---|---|---|
| Harbin | 2010-2013 | 0-14 | 3.59 | 45.8 | Less development |
| Shenyang | 2010-2013 | 0-14 | 2.48 | 41.8 | Less development |
| Beijing | 2010-2013 | 0-14 | 2.46 | 39.9 | Well development |
| Yinchuan | 2010-2013 | 0-14 | 2.43 | 38.5 | Less development |
| Lanzhou | 2010-2013 | 0-14 | 2.22 | 36.0 | Less development |
| Jinan | 2010-2013 | 0-14 | 2.18 | 36.7 | Less development |
| Xi'an | 2010-2013 | 0-14 | 1.82 | 34.3 | Less development |
| Nanjing | 2010-2013 | 0-14 | 2.23 | 32.1 | Well development |
| Shanghai | 1997-2011 | 0-14 | 3.1 | 31.1 | Well development |
| Zhejiang | 2007-2013 | 0-18 | 2.0 | 27.0-31.1 | Well development |
| Chengdu | 2010-2013 | 0-14 | 1.14 | 30.7 | Less development |
| Wuhan | 2010-2013 | 0-14 | 1.61 | 30.5 | Well development |
| Changsha | 2010-2013 | 0-14 | 1.29 | 28.2 | Less development |
| Guangzhou | 2010-2013 | 0-14 | 1.55 | 23.2 | Well development |

GDP: gross domestic product

### Study Limitations

This study has several limitations. First, type 1 and type 2 diabetes in children and adolescents may be misclassified in local hospitals. Second, the limited number of hospitals and cases might cause some missing data and affect the accuracy of incidence. We included the patients in endocrinology department of Children's hospitals and pediatric endocrinology department in general hospitals. However, the missing cases might come from the patients who visited the endocrinology department for adults in local hospitals. We believe the missing cases are limited because of the legal requirements imposed on practicing physician in China. Medical Pracititioners Act in China stipulates only pediatricians can treat children between 0-14 years of age. Third, we didn't collect the clinical characteristics of the patients. According to an earlier survey including patients in Henan province, the majority of patients with T1DM in China have typical symptoms coinciding with the other countries (21).

## Conclusion

In conclusion, this survey was conducted in Henan Province, China, with a total population of nearly 100 million, and covered more than 19 million children years at risk. The incidence rate in Henan Province was 2.19 per 100,000 from 2017 to 2020, with the highest in the 10-14.9 years age group. Additionally, the incidence rate was higher in females than in males, and there were significant differences in the 5-9.9 and 10-14.9 years age groups. The seasonality of the incidence was different from that in previous reports, with the lowest incidence in spring. Finally, the incidence of T1DM in children from Henan Province is still among the lowest reported in the World, according to these results. However, ascertainment is unknown. Therefore, these are minimum estimates and the true incidence may be higher. In future, it would be necessary to promote the establishment of a diabetes registration system to better assess the incidence of diabetes in Henan province.

### Acknowledgments

We thank the hospitals in Henan province that provided hospital registration data.

### Ethics

**Ethics Committee Approval:** The study design was approved by the Ethics Review Board of Children's Hospital Affiliated to Zhengzhou University (protocol no: 2022-K-002, date: 10.01.2017).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Design: Haiyan Wei, Data Collection or Processing: Qiong Chen, Na Xu, Yongxing Chen, Fengyan Tian, Wei Yang, Yan Cui, Ai Huang, Yangshiyu Li, He Zhang, Zhihong Jiang, Ruizhi Zheng, Yuan Ji, Dongming Zhang, Qiao Ren, Li Ding, Haiyan Wei, Analysis or Interpretation: Mingming Yan, Literature Search: Haiyan Wei, Writing: Qiong Chen, Haiyan Wei.

**Financial Disclosure:** The study was supported by Science and Technology Department of Henan Province (project no: 142102310139).

## References

1. Yang Z, Wang K, Li T, Sun W, Li Y, Chang YF, Dorman JS, LaPorte RE. Childhood diabetes in China. Enormous variation by place and ethnic group. Diabetes Care 1998;21:525-529.
2. Weng J, Zhou Z, Guo L, Zhu D, Ji L, Luo X, Mu Y, Jia W; T1D China Study Group. Incidence of type 1 diabetes in China, 2010-13: population based study. BMJ 2018;360:5295.
3. Gong C, Meng X, Jiang Y, Wang X, Cui H, Chen X. Trends in childhood type 1 diabetes mellitus incidence in Beijing from 1995 to 2010: a retrospective multicenter study based on hospitalization data. Diabetes Technol Ther 2015;17:159-165. Epub 2014 Dec 29
4. Tuomilehto J, Ogle GD, Lund-Blix NA, Stene LC. Update on Worldwide Trends in Occurrence of Childhood Type 1 Diabetes in 2020. Pediatr Endocrinol Rev 2020;17(Suppl 1):198-209.
5. Fu JF, Liang L, Gong CX, Xiong F, Luo FH, Liu GL, Li P, Liu L, Xin Y, Yao H, Cui LW, Shi X, Yang Y, Chen LQ, Wei HY. Status and trends of diabetes in Chinese children: analysis of data from 14 medical centers. World J Pediatr 2013;9:127-134. Epub 2013 May 16
6. Wu HB, Zhong JM, Hu RY, Wang H, Gong WW, Pan J, Fei FR, Wang M, Guo LH, Yang L, Yu M. Rapidly rising incidence of Type 1 diabetes in children and adolescents aged 0-19 years in Zhejiang, China, 2007 to 2013. Diabet Med 2016;33:1339-1346. Epub 2015 Nov 22
7. Zhao Z, Sun C, Wang C, Li P, Wang W, Ye J, Gu X, Wang X, Shen S, Zhi D, Lu Z, Ye R, Cheng R, Xi L, Li X, Zheng Z, Zhang M, Luo F. Rapidly rising incidence of childhood type 1 diabetes in Chinese population: epidemiology in Shanghai during 1997-2011. Acta Diabetol 2014;51:947-953. Epub 2014 Apr 29
8. Patterson CC, Gyürüs E, Rosenbauer J, Cinek O, Neu A, Schober E, Parslow RC, Joner G, Svensson J, Castell C, Bingley PJ, Schoenle E, Jarosz-Chobot P, Urbonaité B, Rothe U, Krzisnik C, Ionescu-Tirgoviste C, Weets I, Kocova M, Stipancic G, Samardzic M, de Beaufort CE, Green A, Dahlquist GG, Soltész G. Trends in childhood type 1 diabetes incidence in Europe during 1989-2008: evidence of non-uniformity over time in rates of increase. Diabetologia 2012;55:2142-2147. Epub 2012 May 26.
9. Patterson CC, Dahlquist G, Soltész G, Green A; EURODIAB ACE Study Group. Europe and Diabetes. Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates. Diabetologia 2001;44(Suppl 3):9-16.
10. Soltesz G, Patterson CC, Dahlquist G; EURODIAB Study Group. Worldwide childhood type 1 diabetes incidence--what can we learn from epidemiology? Pediatr Diabetes 2007;8(Suppl 6):6-14.
11. 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
12. Onda Y, Sugihara S, Ogata T, Yokoya S, Yokoyama T, Tajima N; Type 1 Diabetes (T1D) Study Group. Incidence and prevalence of childhood-onset Type 1 diabetes in Japan: the T1D study. Diabet Med 2017;34:909-915. Epub 2017 Feb 2
13. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, Imperatore G, Linder B, Marcovina S, Pettitt DJ, Pihoker C, Saydah S, Wagenknecht L; SEARCH for Diabetes in Youth Study. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. N Engl J Med 2017;376:1419-1429.
14. Nystrom L, Dahlquist G, Ostman J, Wall S, Arnqvist H, Blohme G, Lithner F, Littorin B, Schersten B, Wibell L. Risk of developing insulin-dependent diabetes mellitus (IDDM) before 35 years of age: indications of climatological determinants for age at onset. Int J Epidemiol 1992;21:352-358. Epub 1992 Apr 1
15. Gyürüs E, Györk B, Green A, Patterson C, Soltesz G. [Incidence of type 1 childhood diabetes in Hungary (1978-1997). Hungarian Committee on the Epidemiology of Childhood Diabetes]. Orv Hetil 1999;140:1107-1111.
16. Matsuura N, Fukuda K, Okuno A, Harada S, Fukushima N, Koike A, Ito Y, Hotsubo T. Descriptive epidemiology of IDDM in Hokkaido, Japan: the Childhood IDDM Hokkaido Registry. Diabetes Care 1998;21:1632-1636.
17. Zhang H, Xia W, Yu Q, Wang B, Chen S, Wang Z, Love EJ. Increasing incidence of type 1 diabetes in children aged 0-14 years in Harbin, China (1990-2000). Prim Care Diabetes 2008;2:121-126. Epub 2008 Jul 16
18. Tung JY, Kwan EY, But BW, Wong WH, Fu AC, Pang G, Tsang JW, Yau HC, Belaramani K, Wong LM, Wong SM, Lo P, Ng KL, Yeung WK, Chan KT, Chan AM, Wong SW, Tay MK, Chung J, Lee CY, Lam YY, Cheung PT. Increasing incidence of type 1 diabetes among Hong Kong children and adolescents: The Hong Kong Childhood Diabetes Registry 2008 to 2017. Pediatr Diabetes 2020;21:713-719. Epub 2020 Apr 29
19. Gerasimidi Vazeou A, Kordonouri O, Witsch M, Hermann JM, Forsander G, de Beaufort C, Veeze HJ, Maffeis C, Cherubini V, Cinek O, Piccini B, Holl RW, Danne T; SWEET Study Group. Seasonality at the clinical onset of type 1 diabetes-Lessons from the SWEET database. Pediatr Diabetes 2016;17(Suppl 23):32-37.
20. Ye J, Chen RG, Ashkenazi I, Laron Z. Lack of seasonality in the month of onset of childhood IDDM (0.7-15 years) in Shanghai, China. J Pediatr Endocrinol Metab 1998;11:461-464.
21. Hou L, Li X, Liu L, Wei H, Xiong F, Du H, Yang Y, Zhang H, Zhang Q, Yao H, Fu J, Yan X, Cui L, Liu G, Li T, Chen S, Li P, Xin Y, Liang X, Yu B, Dong Z, Chen R, Ma H, Cheng X, Luo F, Gong C, Song W, Chen X, Zhang Z, Peng X, Li G, Liang L, Maimaiti M, Cheung PT, Luo X. A Multicenter Survey of Type I Diabetes Mellitus in Chinese Children. Front Endocrinol (Lausanne) 2021;12:583114.
22. Jaacks LM, Liu W, Ji L, Mayer-Davis EJ. Type 1 diabetes stigma in China: a call to end the devaluation of individuals living with a manageable chronic disease. Diabetes Res Clin Pract 2015;107:306-307. Epub 2014 Dec 31
23. Li H, Yu G, Duan H, Fu J, Shu Q. Changes in Children's Healthcare Visits During Coronavirus Disease-2019 Pandemic in Hangzhou, China. J Pediatr 2020;224:146-149. Epub 2020 May 19

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2022-3-11

J Clin Res Pediatr Endocrinol 2022;14(3):293-301

# Comparative Analyses of Turkish Variome and Widely Used Genomic Variation Databases for the Evaluation of Rare Sequence Variants in Turkish Individuals: Idiopathic Hypogonadotropic Hypogonadism as a Disease Model

**Leman Damla Kotan**

*Çukurova University Faculty of Medicine, Department of Pediatric Endocrinology, Adana, Turkey*

**What is already known on this topic?**

The absence of population-specific genetic variation reference databases causes misleading results in rare variant evaluations.

**What this study adds?**

This study confirmed that Turkish Variome could represent the Turkish population for rare genomic variant evaluation.

## Abstract

**Objective:** With the increasing use of whole-exome sequencing, one of the challenges in identifying the causal allele for a Mendelian disease is the lack of availability of population-specific human genetic variation reference databases. The people of Turkey were not represented in GnomAD or other publicly available large databases until recently, when the first comprehensive genomic variation database, Turkish Variome (TRV), was published. The aim of this study was to evaluate whether TRV or other publicly available large genomic variation databases can reliably be used for rare disease variant evaluation in Turkish individuals.

**Methods:** Sixty non-disease-causing, non-synonymous variants (minor allele frequencies >1%) were identified in 58 genes that are known to be associated with idiopathic hypogonadotropic hypogonadism from a large Turkish patient cohort. The allelic frequencies of these variants were then compared with those in various public genomic variation databases, including TRV.

**Results:** Our cohort variants showed the highest correlations with those in the TRV, Iranome, and The Greater Middle East Variome, in decreasing order.

**Conclusion:** These results suggest that the TRV is the appropriate database to use for rare genomic variant evaluations in the Turkish population. Our data also suggest that variomes from geographic neighborhoods may serve as substitute references for populations devoid of their own genomic variation databases.

**Keywords:** Allele frequency, Turkish Variome, variant evaluation, genomic variation databases

## Introduction

The widespread use of next-generation sequencing (NGS), particularly whole-exome sequencing (WES), in medical practice, has resulted in massive data accumulation (1). In order to accurately interpret the differences in the DNA sequences of individuals, criteria based on specific parameters are used. One of the essential parameters is allele frequency (AF), which represents the prevalence of a gene variant in a given population. Variants with minor AFs less than 1% are considered rare and can play a causative role in Mendelian and complex disorders. Genetic alterations observed with a much higher frequency than expected for the disease in a population are generally interpreted as benign (2,3). As many variants are proven to be population-specific, large databases evolved into a comprehensive body of data comprising of datasets from individual subpopulations

**Address for Correspondence:** Leman Damla Kotan MD, Çukurova University Faculty of Medicine, Department of Pediatric Endocrinology, Adana, Turkey
**E-mail:** damlakotan@gmail.com, dkotan@cu.edu.tr **ORCID:** orcid.org/0000-0001-6176-8986

**Conflict of interest:** None declared
**Received:** 14.03.2022
**Accepted:** 06.04.2022

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

(4). Failure to use population-specific databases can lead to unreliable or even misleading results in variant evaluation.

The people of Turkey live in the Anatolian peninsula, which is geographically at the crossroads of three major continents, through which major population movements have occurred during all periods of human history. Therefore, it was thought that this geographic region might have a genetic admixture. The genetic structure of Turkish people has been investigated in small scale studies using different methods (5,6,7,8). In a recent study, Kars et al. (9) published the first comprehensive genomic variation database, Turkish Variome (TRV), which compiles whole genome and whole exome data from 3362 individuals from various regions of Turkey.

The aim of this study was to evaluate whether any large population variomes, including TRV, can reliably be used in variant evaluations for the population of Turkey. Therefore, 60 non-disease-causing non-synonymous variants (minor AFs greater than 1%) in 58 genes, known to be associated with idiopathic hypogonadotropic hypogonadism (IHH), were compared with the AFs in various population databases worldwide.

## Methods

### Patient Cohort

The study used genetic variants from a large, rare disease cohort. The cohort included a total of 290 independent patients (112 female and 178 male) from seven geographic regions of Turkey (the Marmara region, Black Sea, Aegean, Mediterranean, Central Anatolia, Eastern Anatolia, and Southeastern Anatolia), roughly representing the population of Turkey.

### Disease Model

IHH is a rare disease characterized by pubertal failure and infertility, with a prevalence of 1/10-100.000 (10). Mutations detected in patients with IHH, based on the Mendelian inheritance model, are currently thought to be responsible for approximately 50% of all cases (11).

### Genetic Analyses

A total of 290 WES data sets were screened for potentially pathogenic nucleotide changes [frameshifts, in-frame changes (insertion and deletion), nonsense (stop-loss and stop-gain), two-base splice-sites (donor/acceptor), and missense] located in the exons of 58 genes known to be associated with IHH. Intronic areas, distant regions, and synonymous changes were excluded. Currently known-IHH-associated genes are listed in Table 1 (11).

### Selection of the Study Variants

Based on the prevalence of IHH (1/10.100.000), those variants with an AF lower than 0.0001 were excluded from the study as they can be of high pathogenicity. We also excluded those that can be potentially pathogenic with an AF of 0.01-0.0001. In this study, we only included those with AF greater than 0.01, which are extremely unlikely to be disease-causing for IHH.

### WES Analyses

Briefly, the genomic DNA samples from each patient were prepared as an Illumina sequencing library. Afterward, sequencing libraries were enriched for proper targets with the Illumina Exome Enrichment protocol. Captured libraries were sequenced with Illumina HiSeq 2000 Sequencer (Macrogen, Seoul, South Korea). The reads were mapped to UCSC hg19.

### Databases

The seven established databases used for the AF correlations with our cohort were: GnomAD, which includes European Finnish, Europen Non-Finnish (ENF), Ashkenazi Jewish, East Asian, South Asian, Latino/Admixed American, and African/African-American subcategories (12); The NHLBI Trans-Omics for Precision Medicine representing a diverse population around the world with multi-ethnic data content (European, Hispanic/Latino, African, Asian) (13); The Greater Middle East (GME) Variome Project, which includes the GME world population, from Morocco in the west to Pakistan in the East including 163 alleles from the Turkish peninsula (14); Iranome, which includes Iranian Arabs, Kurds, Persians, Persian Gulf Islanders, Azeris, and Turkmen ethnic groups (15); GenomeAsia, which includes South East Asian, Oceania, North East Asian, African, West Eurasia, South Asian, and American subpopulations (16); the 4.7KJPN, which represents the overall Japanese population (17); and Online Archive of Brazilian Mutations, which includes Brazilian population (18). The GnomAD ENF category includes Southern European, Bulgarian, North-Western European, Swedish, and Estonian subpopulations. Categories named as "others: were not included in the study. The AFs were collected from the databases in February 2022. URLs of databases are provided in the web resources.

### Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, version 20.0 (IBM Inc., Armonk, NY, USA), and a p value of $< 0.001$ was considered

**Table 1. Genes known to be associated with idiopathic hypogonadotropic hypogonadism**

| Approved symbol | Approved name | HGNC ID | Chromosomal location |
|---|---|---|---|
| *AMH* | anti-Mullerian hormone | 464 | 19p13.3 |
| *AMHR2* | anti-Mullerian hormone receptor type 2 | 465 | 12q13.13 |
| *ANOS1* | anosmin 1 | 6211 | Xp22.31 |
| *AXL* | AXL receptor tyrosine kinase | 905 | 19q13.2 |
| *CCDC141* | coiled-coil domain containing 141 | 26821 | 2q31.2 |
| *CHD7* | chromodomain helicase DNA binding protein 7 | 20626 | 8q12.2 |
| *DCC* | DCC netrin 1 receptor | 2701 | 18q21.2 |
| *DLG2* | discs large MAGUK scaffold protein 2 | 2901 | 11q14.1 |
| *DMXL2* | Dmx like 2 | 2938 | 15q21.2 |
| *DUSP6* | dual specificity phosphatase 6 | 3072 | 12q21.33 |
| *FEZF1* | FEZ family zinc finger 1 | 22788 | 7q31.32 |
| *FGF17* | fibroblast growth factor 17 | 3673 | 8p21.3 |
| *FGF8* | fibroblast growth factor 8 | 3686 | 10q24.32 |
| *FGFR1* | fibroblast growth factor receptor 1 | 3688 | 8p11.23 |
| *FLRT3* | fibronectin leucine rich transmembrane protein 3 | 3762 | 20p12.1 |
| *FSHB* | follicle stimulating hormone subunit beta | 3964 | 11p14.1 |
| *GNRH1* | gonadotropin releasing hormone 1 | 4419 | 8p21.2 |
| *GNRHR* | gonadotropin releasing hormone receptor | 4421 | 4q13.2 |
| *HESX1* | HESX homeobox 1 | 4877 | 3p14.3 |
| *HS6ST1* | heparan sulfate 6-O-sulfotransferase 1 | 5201 | 2q14.3 |
| *IGSF10* | immunoglobulin superfamily member 10 | 26384 | 3q25.1 |
| *IL17RD* | interleukin 17 receptor D | 17616 | 3p14.3 |
| *KISS1* | KiSS-1 metastasis suppressor | 6341 | 1q32.1 |
| *KISS1R* | KISS1 receptor | 4510 | 19p13.3 |
| *KLB* | klotho beta | 15527 | 4p14 |
| *LEP* | leptin | 6553 | 7q32.1 |
| *LEPR* | leptin receptor | 6554 | 1p31.3 |
| *LHB* | luteinizing hormone subunit beta | 6584 | 19q13.33 |
| *NDNF* | neuron derived neurotrophic factor | 26256 | 4q27 |
| *NR0B1* | nuclear receptor subfamily 0 group B member 1 | 7960 | Xp21.2 |
| *NSMF* | NMDA receptor synaptonuclear signaling and neuronal migration factor | 29843 | 9q34.3 |
| *NTN1* | netrin 1 | 8029 | 17p13.1 |
| *OTUD4* | OTU deubiquitinase 4 | 24949 | 4q31.21 |
| *PCSK1* | proprotein convertase subtilisin/kexin type 1 | 8743 | 5q15 |
| *PLXNA1* | plexin A1 | 9099 | 3q21.3 |
| *PLXNA3* | plexin A3 | 9101 | Xq28 |
| *PNPLA6* | patatin like phospholipase domain containing 6 | 16268 | 19p13.2 |
| *POLR3A* | RNA polymerase III subunit A | 30074 | 10q22.3 |
| *POLR3B* | RNA polymerase III subunit B | 30348 | 12q23.3 |
| *PROK2* | prokineticin 2 | 18455 | 3p13 |
| *PROKR2* | prokineticin receptor 2 | 15836 | 20p12.3 |
| *RAB18* | RAB18, member RAS oncogene family | 14244 | 10p12.1 |
| *RAB3GAP1* | RAB3 GTPase activating protein catalytic subunit 1 | 17063 | 2q21.3 |
| *RAB3GAP2* | RAB3 GTPase activating non-catalytic protein subunit 2 | 17168 | 1q41 |
| *RNF216* | ring finger protein 216 | 21698 | 7p22.1 |
| *SEMA3A* | semaphorin 3A | 10723 | 7q21.11 |
| *SEMA3E* | semaphorin 3E | 10727 | 7q21.11 |

J Clin Res Pediatr Endocrinol
2022;14(3):293-301

**Table 1. Continued**

| Approved symbol | Approved name | HGNC ID | Chromosomal location |
|---|---|---|---|
| *SEMA3F* | semaphorin 3F | 10728 | 3p21.31 |
| *SMCHD1* | structural maintenance of chromosomes flexible hinge domain containing 1 | 29090 | 18p11.32 |
| *SOX10* | SRY-box transcription factor 10 | 11190 | 22q13.1 |
| *SPRY4* | sprouty RTK signaling antagonist 4 | 15533 | 5q31.3 |
| *SRA1* | steroid receptor RNA activator 1 | 11281 | 5q31.3 |
| *STUB1* | STIP1 homology and U-box containing protein 1 | 11427 | 16p13.3 |
| *TAC3* | tachykinin precursor 3 | 11521 | 12q13.3 |
| *TACR3* | tachykinin receptor 3 | 11528 | 4q24 |
| *TBC1D20* | TBC1 domain family member 20 | 16133 | 20p13 |
| *TUBB3* | tubulin beta 3 class III | 20772 | 16q24.3 |
| *WDR11* | WD repeat domain 11 | 13831 | 10q26.12 |

HGNC: https://www.genenames.org, HGNC: HUGO Gene Nomenclature Committee

**Table 2. The allelic frequencies (AF) of the variants in the IHH cohort with greater than 1% and AF values in selected databases**

| Gene | dbSNP | Cohort AF | TRV AF | Genome Asia AF | GnomAD AF | GME AF | ToPMed Bravo AF | Iranome AF | 4.7KJPN AF | ABraOM |
|---|---|---|---|---|---|---|---|---|---|---|
| *AXL* | rs7249222 | 0.9844 | 1.0000 | 1.0000 | 1.0000 | - | 1.0000 | 1.0000 | 0.0000 | 1.0000 |
| *DCC* | rs9951523 | 0.9810 | 0.9886 | 0.9980 | 0.9853 | 0.9914 | 0.9913 | 0.9919 | 1.0000 | 0.9885 |
| *AMH* | rs10417628 | 0.9542 | 0.9782 | 0.9890 | 0.9817 | 0.9777 | 0.9834 | 0.9794 | 0.9999 | 0.9926 |
| *AMH* | rs10407022 | 0.8224 | 0.8091 | 0.6480 | 0.7629 | 0.7773 | 0.6976 | 0.7956 | 0.6523 | 0.7775 |
| *IGSF10* | rs7619322 | 0.7948 | 0.7848 | 0.7390 | 0.7196 | 0.8145 | 0.6976 | 0.7937 | 0.6613 | 0.7471 |
| *SEMA3F* | rs1046956 | 0.7000 | 0.7164 | 0.9150 | 0.7347 | 0.6491 | 0.6479 | 0.7406 | 0.9999 | 0.6789 |
| *IL17RD* | rs6780995 | 0.6931 | 0.6630 | 0.4160 | 0.6201 | 0.6500 | 0.6218 | 0.6981 | 0.1611 | 0.6362 |
| *KISS1R* | rs350132 | 0.6775 | 0.7823 | 0.7630 | 0.7942 | 0.8127 | 0.8204 | 0.7432 | 0.7679 | 0.7830 |
| *SMCHD1* | rs2276092 | 0.6396 | 0.6456 | 0.6540 | 0.6960 | 0.7134 | 0.7388 | 0.6625 | 0.5547 | 0.7348 |
| *ANOS1* | rs808119 | 0.5970 | 0.6157 | - | 0.5590 | 0.7189 | 0.5325 | 0.5381 | 0.6755 | 0.5450 |
| *SRA1* | rs5871740 | 0.4879 | 0.5120 | 0.4660 | 0.1400 | 0.4506 | - | 0.5331 | 0.5113 | - |
| *DMXL2* | rs12102203 | 0.4586 | 0.4670 | 0.4180 | 0.4936 | 0.4526 | 0.4850 | 0.4544 | 0.4661 | 0.5090 |
| *DUSP6* | rs2279574 | 0.4344 | 0.4683 | 0.5590 | 0.5177 | 0.4558 | 0.4200 | 0.4719 | 0.5175 | 0.4367 |
| *SRA1* | rs3085220 | 0.4224 | 0.5120 | 0.4660 | 0.1221 | - | - | 0.5331 | 0.5113 | 0.1976 |
| *DCC* | rs2229080 | 0.4034 | 0.4611 | - | 0.4416 | 0.4879 | 0.3782 | 0.4888 | 0.6595 | 0.4293 |
| *RAB3GAP1* | rs10445686 | 0.3862 | 0.3843 | 0.3210 | 0.1973 | 0.3524 | 0.1264 | 0.3919 | 0.4118 | 0.2348 |
| *HS6ST1* | rs200979099 | 0.3672 | - | - | 0.3773 | 0.4030 | 0.0907 | 0.4381 | 0.0144 | 0.3801 |
| *FLRT3* | rs6079391 | 0.3568 | 0.3694 | 0.2380 | 0.4098 | 0.2754 | 0.3375 | 0.3325 | 0.1492 | 0.3924 |
| *PLXNA3* | rs5945430 | 0.3383 | 0.3038 | - | 0.2148 | 0.3668 | 0.6666 | 0.4113 | 0.0524 | 0.2784 |
| *LEPR* | rs1137101 | 0.3241 | 0.3532 | 0.6800 | 0.5089 | 0.3487 | 0.4871 | 0.3394 | 0.8621 | 0.4384 |
| *SMCHD1* | rs633422 | 0.3068 | 0.3236 | - | 0.3383 | - | 0.2502 | 0.2781 | 0.2417 | 0.3481 |
| *KISS1* | rs4889 | 0.3051 | 0.3186 | 0.3080 | 0.2874 | 0.3132 | 0.3238 | 0.2932 | 0.4662 | 0.3001 |
| *KLB* | rs4975017 | 0.2862 | 0.2897 | 0.4350 | 0.3428 | 0.2275 | 0.2525 | 0.2938 | 0.3801 | 0.2889 |
| *PCSK1* | rs6234 | 0.2517 | 0.2725 | 0.2800 | 0.2633 | 0.2573 | 0.2263 | 0.2662 | 0.2093 | 0.2430 |
| *PCSK1* | rs6235 | 0.2466 | 0.2719 | 0.2780 | 0.2595 | 0.2532 | 0.2110 | 0.2631 | 0.2094 | 0.2356 |
| *PNPLA6* | rs17854645 | 0.2362 | 0.2403 | 0.0820 | 0.1620 | 0.2152 | 0.1479 | 0.2459 | 0.0001 | 0.1674 |
| *KISS1* | rs71745629 | 0.2292 | 0.2794 | 0.2750 | 0.2230 | - | 0.1705 | 0.2214 | 0.4625 | 0.2059 |
| *DMXL2* | rs17524906 | 0.2224 | 0.2151 | 0.0760 | 0.1819 | 0.2064 | 0.1806 | 0.2156 | 0.0119 | 0.1962 |
| *LEPR* | rs1805094 | 0.2000 | 0.2496 | 0.0970 | 0.1584 | - | 0.1822 | 0.2662 | 0.1010 | 0.1937 |
| *KLB* | rs17618244 | 0.1965 | 0.2182 | 0.1750 | 0.1793 | 0.2139 | 0.1357 | 0.1994 | 0.1838 | 0.1929 |
| *HS6ST1* | rs3958533 | 0.1931 | - | - | 0.1193 | 0.3171 | 0.1031 | 0.4206 | 0.0214 | 0.2529 |
| *CCDC141* | rs61397643 | 0.1896 | 0.1642 | 0.1250 | 0.1310 | 0.1614 | 0.1165 | 0.1950 | 0.1172 | 0.1403 |

**Table 2. Continued**

| Gene | dbSNP | Cohort AF | TRV AF | Genome Asia AF | GnomAD AF | GME AF | ToPMed Bravo AF | Iranome AF | 4.7KJPN AF | ABraOM |
|---|---|---|---|---|---|---|---|---|---|---|
| *GNRH1* | rs6185 | 0.1793 | 0.1926 | 0.2920 | 0.2299 | 0.1596 | 0.1844 | 0.1725 | 0.5192 | 0.1945 |
| *HS6ST1* | rs199993343 | 0.1727 | - | - | 0.0898 | 0.1554 | 0.0222 | 0.3302 | 0.0081 | 0.0371 |
| *IL17RD* | rs17057718 | 0.1706 | 0.1848 | 0.3050 | 0.1983 | 0.1898 | 0.1500 | 0.1931 | 0.3382 | 0.1724 |
| *LEPR* | rs1137100 | 0.1379 | 0.1771 | 0.4140 | 0.2959 | 0.1077 | 0.2148 | 0.1556 | 0.7813 | 0.2298 |
| *DUSP6* | rs770087 | 0.1327 | 0.1613 | 0.1230 | 0.1960 | 0.1930 | 0.2890 | 0.1537 | 0.0388 | 0.2578 |
| *SEMA3E* | rs61729612 | 0.1224 | 0.1215 | 0.1066 | 0.1161 | 0.1406 | 0.1022 | 0.1144 | 0.2172 | 0.1215 |
| *RAB3GAP2* | rs2289189 | 0.1120 | 0.1376 | 0.0840 | 0.0840 | 0.0936 | 0.0603 | 0.1206 | 0.0809 | 0.0853 |
| *CCDC141* | rs34883828 | 0.0931 | 0.1254 | 0.0560 | 0.1070 | 0.1168 | 0.1294 | 0.1044 | 0.0097 | 0.1124 |
| *CCDC141* | rs12988301 | 0.0862 | 0.1097 | 0.1010 | 0.0832 | 0.1072 | 0.1194 | 0.0887 | 0.1557 | 0.1100 |
| *IGSF10* | rs12487205 | 0.0810 | 0.0792 | 0.0320 | 0.0341 | 0.0694 | 0.0358 | 0.0818 | 0.0413 | 0.0517 |
| *IGSF10* | rs17204557 | 0.0810 | 0.0772 | 0.0690 | 0.0416 | 0.0720 | 0.0310 | 0.0806 | 0.0409 | 0.0476 |
| *CCDC141* | rs17362588 | 0.0637 | 0.0546 | 0.0110 | 0.0604 | 0.0629 | 0.0452 | 0.0618 | - | 0.0689 |
| *RAB3GAP2* | rs12045447 | 0.0586 | 0.0463 | 0.0470 | 0.0390 | 0.0808 | 0.0587 | 0.0481 | 0.0326 | 0.0418 |
| *PCSK1* | rs6232 | 0.0534 | 0.0547 | 0.0210 | 0.0381 | 0.0523 | 0.0290 | 0.0518 | 0.0003 | 0.0287 |
| *POLR3B* | rs17038460 | 0.0517 | 0.0612 | 0.0100 | 0.0460 | 0.0634 | 0.0410 | 0.0550 | - | 0.0870 |
| *LHB* | rs1800447 | 0.0465 | 0.0621 | - | 0.0665 | 0.0888 | 0.0768 | 0.0381 | 0.0452 | 0.0343 |
| *LHB* | rs34349826 | 0.0431 | 0.0610 | - | 0.0522 | 0.0833 | - | 0.0375 | 0.0452 | 0.0269 |
| *CCDC141* | rs13419085 | 0.0431 | 0.0412 | 0.0900 | 0.0606 | 0.0453 | 0.0266 | 0.0600 | 0.0166 | 0.0492 |
| *HS6ST1* | rs201154532 | 0.0413 | 0.0442 | - | 0.0541 | 0.0229 | 0.0443 | 0.0538 | 0.0060 | 0.0345 |
| *KISS1* | rs12998 | 0.0327 | 0.0365 | 0.0360 | 0.0325 | 0.0483 | 0.0241 | 0.0393 | 0.0375 | 0.0377 |
| *KISS1* | rs35431622 | 0.0275 | 0.0422 | 0.0021 | 0.0518 | 0.0724 | 0.1207 | 0.0487 | - | 0.0812 |
| *KLB* | rs35372803 | 0.0275 | 0.0213 | 0.0066 | 0.0362 | 0.0186 | 0.0292 | 0.0150 | 0.0028 | 0.0213 |
| *SEMA3A* | rs147436181 | 0.0275 | 0.0180 | 0.0057 | 0.0137 | 0.0176 | 0.0106 | 0.0275 | - | 0.0188 |
| *DUSP6* | rs61734372 | 0.0241 | 0.0200 | 0.0046 | 0.0238 | 0.0121 | 0.0201 | 0.0156 | - | 0.0246 |
| *FLRT3* | rs35253731 | 0.0206 | 0.0209 | 0.0232 | 0.0319 | 0.0382 | 0.0669 | 0.0218 | 0.0124 | 0.0402 |
| *OTUD4* | rs36225838 | 0.0206 | 0.0261 | 0.0054 | 0.0256 | 0.0282 | 0.0221 | 0.0243 | - | 0.0336 |
| *STUB1* | rs148553428 | 0.0120 | 0.0064 | 0.0046 | 0.0037 | 0.0065 | 0.0024 | 0.0093 | - | 0.0016 |
| *IGSF10* | rs34114908 | 0.0189 | 0.0110 | 0.0017 | 0.0127 | 0.0120 | 0.0084 | 0.0081 | - | 0.0147 |

Variants are arranged by allelic frequency from high to low.
dbSNP: Database of Single Nucleotide Polymorphisms, TRV: Turkish Variome, GME: The Greater Middle East Variom Project, GnomAD: genome aggregation database, TopMeD Bravo: Trans-Omics for Precision Medicine, ToMMo-4.7KJPN: Tohoku Medical Megabank Organization, ABraOM: Online Archive of Brazilian Mutations, IHH: idiopathic hypogonadotropic hypogonadism, -: absent, GME: http://igm.ucsd.edu/gme/, TopMED Bravo: https://bravo.sph.umich.edu/freeze5/hg38/, ToMMo-4.7KJPN: https://jmorp.megabank.tohoku.ac.jp/202102/, ABraOM: https://abraom.ib.usp.br

statistically significant. The Spearman's correlation method was used as the variables in the comparison of the groups were non-normally distributed. The correlation coefficients (CCs) between the study cohort and each of the databases/subgroups were analyzed separately. All correlation analysis results were found to be statistically significant. Next, we compared the CCs based on the concept of comparison of correlations from independent samples.

## Results

In this study, a total of 60 variants with an AF greater than 1% were detected in 30 of 58 IHH-associated genes in the WES data from the cohort of 290 independent Turkish IHH patients (Table 2). No variants above the cut-off were observed in 26 of the listed IHH genes, while 17 genes had more than one (maximum five) variant and 13 genes had only one. The great majority of the changes (95.0%) were missense, and 5.0% were frameshift (two insertions and one deletion). Each of the variants in the study cohort was observed only in the Iranome and GnomAD.

A statistically significant correlation was observed between the study cohort and each one of the databases analyzed (Table 3). The highest CCs were observed between the study cohort and the following databases, in decreasing order: TRV (0.994), Iranome (0.983), and GME (0.981). Comparison of correlations from independent samples indicated that the

CCs of these three databases with our study cohort were not statistically different from each other (Table 3, shown in bold). The remaining 32 CCs were significantly different. Thus, the comparison results were interpreted as such that the three databases (TRV, Iranome, and GME) can be used as the reference databases for Turkish individuals.

## Discussion

Population studies have repeatedly revealed the importance of local datasets in research and clinical practice, rather than using comprehensive databases with a wide-ranging sample size (4,19,20). Knowing the AF differences between

**Table 3. The correlation coefficients (CCs) between the IHH cohort and various databases**

| Databases | Total number of 60 variants encountered | Correlation coefficients |
|---|---|---|
| **TurkishVariome** | **57** | **0.994** |
| **Iranome** | **60** | **0.983** |
| **The Greater Middle East Variom Project (GME)** | **55** | **0.981** |
| Arab# | 60 | 0.978 |
| Azeri# | 60 | 0.976 |
| Persian# | 60 | 0.975 |
| West Eurasia* | 50 | 0.974 |
| Lur# | 60 | 0.969 |
| Kurd# | 60 | 0.968 |
| Turkmen# | 60 | 0.963 |
| Persian Gulf Islander# | 60 | 0.958 |
| Southern European +, ~ | 60 | 0.955 |
| Online Archive of Brazilian Mutations (ABraOM) | 59 | 0.951 |
| South Asian* | 50 | 0.949 |
| Baloch# | 60 | 0.948 |
| Bulgarian +, ~ | 60 | 0.943 |
| GenomeAsia | 50 | 0.940 |
| GnomAD | 60 | 0.935 |
| European Non-Finnish + | 60 | 0.923 |
| South Asian + | 60 | 0.920 |
| Ashkenazi Jewish + | 60 | 0.920 |
| Latino/Admixed American + | 60 | 0.913 |
| North-Western European +, ~ | 60 | 0.911 |
| Swedish +, ~ | 60 | 0.907 |
| Trans-Omics for Precision Medicine (TopMed) | 57 | 0.906 |
| North East Asian* | 50 | 0.892 |
| European Finnish + | 60 | 0.886 |
| American* | 50 | 0.868 |
| Estonian +, ~ | 60 | 0.867 |
| South East Asian* | 50 | 0.861 |
| East Asian + | 60 | 0.849 |
| Oceania* | 50 | 0.841 |
| African/African-American + | 60 | 0.817 |
| African* | 50 | 0.802 |
| Tohoku Medical Megabank Organization (4.7KJPN) | 52 | 0.639 |

The Spearman's Correlation method was used for non-normally distributed variables in the comparison of the groups. Statistical analyses were performed using SPSS 20.0 and a p-value of $< 0.001$ was considered statistically significant. The correlation coefficients (CCs) between the study cohort and each of the databases/subgroups were analyzed separately. All correlation analysis results were found to be statistically significant. The databases/subgroups were arranged by CCs from high to low. Comparison of correlations from independent samples indicated that the CC of these three databases with our study cohort were not statistically different from each other (shown in bold). Symbols indicate from which database the subgroups were collected; *: GenomeAsia, +: GnomAD (Genome aggregation database), ~: GnomAD ENF (European Non- Finnish), #: Iranome.
gnomAD: https://gnomad.broadinstitute.org, GenomeAsia: https://browser.genomeasia100k.org, Iranome: http://www.iranome.ir, ABraOM: https://abraom.ib.usp.br, IHH: idiopathic hypogonadotropic hypogonadism

populations is also essential for developing machine-learning-based methods that use clustering scores for pathogenicity classifications (21). Disease genetics studies in a given population may also provide information for community characteristics, such as mutation history, local adaptations, and avoiding false-positive genetic diagnoses of Mendelian disorders. In this way, identifying and labeling population-specific genetic changes, such as individual/family-specific variants, will significantly reduce the burden of variants of uncertain significance (22,23). In our study, the common variants of Turkish IHH patients were observed at varying frequencies in different populations, supporting the hypothesis that a population-specific reference database should be used to facilitate the selection of pathogenic variants.

It is essential to understand that common and rare alleles have different characteristics. A rare variation is needed to survive many generations to rise to a moderate frequency, while common ones tend to be inherited over long periods due to negligible effects and are most likely classified as benign. Thus, they are excellent candidates for determining demographic histories or periodical features, such as ancestral origins and migration routes (24,25,26). Blekhman et al. (27) observed that Mendelian-disease gene variants, in general, are under purifying selection pressures. The IHH-associated gene variants should be expected to be subjected to additional negative selection pressures as pathogenic variants in these genes result in infertility. This reproductive disadvantage causes them to be rapidly purged from the population (27). Consequently, the AFs of the IHH gene variants are expected to be more skewed compared to most of the Mendelian disease genes except for those with very high mortality. However, common variants (minor allele frequencies $>1\%$) are free of such distortions. Based on the foregoing argument, we selectively compared the common variants in the IHH-related genes with those of the TRV and other publically available databases. Our cohort results showed a nearly one-to-one correlation (0.994) with TRV, which is comprised of NGS data from individuals participating in genetic studies of various diseases, such as obesity, amyotrophic lateral sclerosis, and Parkinson's disease. Our study using a rare disease model, IHH, which is not represented in the TRV patient subpopulations, confirms that TRV is well representative of the Turkish population. Kars et al. (9) also reported the close genetic relationship between Balkan and Caucasian populations and those of Turkey. Previously, similar to our methods paradigm, Alkan et al. (5) studied the 16 genomes from various regions of Turkey and compared them to those in the 1000 Genomes Project, and showed that the genetic structure of the people of Turkey is similar to those of Europe, particularly the Southern Europe/Mediterranean region, compared to other gene pools. Similarly, in our study, a close correlation, albeit to a lesser extent, was also observed with those of West Eurasia, including Caucasia (0.974) and Southern Europe (0.955).

It is well-known that consanguineous unions increase the incidence of recessively inherited diseases (28). Our study included 290 independent IHH patients, and consanguinity was present in 56.0%. This rate is higher than the general Turkish population (21.1%), probably due to a rare disease that could be recessively inherited (29). Studies have reported that high consanguineous marriage is common in many regions, including Turkey, Iran, and Pakistan (29,30,31). The kinship union is influenced by culture, religion, geographical conditions, or socioeconomic boundaries. The AFs in our study cohort did not show remarkable similarity for those in different databases in distant geographies. However, the close correlations with the non-European neighbors of the Anatolian peninsula, Iranome (0.983) and GME (0.981) suggest our genetic similarity for alleles that are relatively difficult to spread due to this social structure (28).

### Study Limitations

The use of WES analyses performed at different periods in the study may have resulted in differences between reads that confidently support alleles.

## Conclusion

Our findings confirm that TRV can be reliably used for variant evaluations from the Turkish population. Our results also indicate that variomes from geographic neighborhoods may serve as substitute references in variant evaluation for populations devoid of representative databases.

### Acknowledgements

I am grateful to Prof. Dr. A. Kemal Topaloğlu for his valuable comments and continuing support of this project. I want to thank Prof. Dr. Ayşe Serin and Dr. Hüsniye Canan for useful discussions. I also thank Assoc. Prof. Dr. İhsan Turan for helping with statistical analyses.

### Ethics

**Ethics Committee Approval:** The Ethics Committee of the Çukurova University Faculty of Medicine approved this study (decision no: 47, date: 02.11.2018).

**Informed Consent:** Informed consent form was obtained from all patients and/or their representative.

**Peer-review:** Externally and internally peer-reviewed.

**Financial Disclosure:** This work was supported by the Çukurova University Scientific Research project number: 11364.

## References

1. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA, Eng CM. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med 2013;369:1502-1511. Epub 2013/10/04
2. 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, Committee ALQA. 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 2015/03/06
3. Ku CS, Loy EY, Salim A, Pawitan Y, Chia KS. The discovery of human genetic variations and their use as disease markers: past, present and future. J Hum Genet 2010;55:403-415. Epub 2010/05/21
4. van Rooij JGJ, Jhamai M, Arp PP, Nouwens SCA, Verkerk M, Hofman A, Ikram MA, Verkerk AJ, van Meurs JBJ, Rivadeneira F, Uitterlinden AG, Kraaij R. Population-specific genetic variation in large sequencing data sets: why more data is still better. Eur J Hum Genet 2017;25:1173-1175. Epub 2017/09/15
5. Alkan C, Kavak P, Somel M, Gokcumen O, Ugurlu S, Saygi C, Dal E, Bugra K, Gungor T, Sahinalp SC, Ozoren N, Bekpen C. Whole genome sequencing of Turkish genomes reveals functional private alleles and impact of genetic interactions with Europe, Asia and Africa. BMC Genomics 2014;15:963. Epub 2014/11/08
6. Hodoglugil U, Mahley RW. Turkish population structure and genetic ancestry reveal relatedness among Eurasian populations. Ann Hum Genet 2012;76:128-141. Epub 2012/02/16
7. Cinnioglu C, King R, Kivisild T, Kalfoglu E, Atasoy S, Cavalleri GL, Lillie AS, Roseman CC, Lin AA, Prince K, Oefner PJ, Shen P, Semino O, Cavalli-Sforza LL, Underhill PA. Excavating Y-chromosome haplotype strata in Anatolia. Hum Genet 2004;114:127-148. Epub 2003/10/31
8. Di Benedetto G, Erguven A, Stenico M, Castri L, Bertorelle G, Togan I, Barbujani G. DNA diversity and population admixture in Anatolia. Am J Phys Anthropol 2001;115:144-156. Epub 2001/06/01
9. Kars ME, Basak AN, Onat OE, Bilguvar K, Choi J, Itan Y, Caglar C, Palvadeau R, Casanova JL, Cooper DN, Stenson PD, Yavuz A, Bulus H, Gunel M, Friedman JM, Ozcelik T. The genetic structure of the Turkish population reveals high levels of variation and admixture. Proc Natl Acad Sci U S A 2021;118. Epub 2021/08/25
10. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 2009;5:569-576. Epub 2009/08/27
11. Topaloglu AK, Turan I. Genetic Etiology of Idiopathic Hypogonadotropic Hypogonadism. Endocrines 2022;3:1-15.
12. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-291. Epub 2016/08/19
13. Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, Taliun SAG, Corvelo A, Gogarten SM, Kang HM, Pitsillides AN, LeFaive J, Lee SB, Tian X, Browning BL, Das S, Emde AK, Clarke WE, Loesch DP, Shetty AC, Blackwell TW, Smith AV, Wong Q, Liu X, Conomos MP, Bobo DM, Aguet F, Albert C, Alonso A, Ardlie KG, Arking DE, Aslibekyan S, Auer PL, Barnard J, Barr RG, Barwick L, Becker LC, Beer RL, Benjamin EJ, Bielak LF, Blangero J, Boehnke M, Bowden DW, Brody JA, Burchard EG, Cade BE, Casella JF, Chalazan B, Chasman DI, Chen YI, Cho MH, Choi SH, Chung MK, Clish CB, Correa A, Curran JE, Custer B, Darbar D, Daya M, de Andrade M, DeMeo DL, Dutcher SK, Ellinor PT, Emery LS, Eng C, Fatkin D, Fingerlin T, Forer L, Fornage M, Franceschini N, Fuchsberger C, Fullerton SM, Germer S, Gladwin MT, Gottlieb DJ, Guo X, Hall ME, He J, Heard-Costa NL, Heckbert SR, Irvin MR, Johnsen JM, Johnson AD, Kaplan R, Kardia SLR, Kelly T, Kelly S, Kenny EE, Kiel DP, Klemmer R, Konkle BA, Kooperberg C, Kottgen A, Lange LA, Lasky-Su J, Levy D, Lin X, Lin KH, Liu C, Loos RJF, Garman L, Gerszten R, Lubitz SA, Lunetta KL, Mak ACY, Manichaikul A, Manning AK, Mathias RA, McManus DD, McGarvey ST, Meigs JB, Meyers DA, Mikulla JL, Minear MA, Mitchell BD, Mohanty S, Montasser ME, Montgomery C, Morrison AC, Murabito JM, Natale A, Natarajan P, Nelson SC, North KE, O'Connell JR, Palmer ND, Pankratz N, Peloso GM, Peyser PA, Pleiness J, Post WS, Psaty BM, Rao DC, Redline S, Reiner AP, Roden D, Rotter JI, Ruczinski I, Sarnowski C, Schoenherr S, Schwartz DA, Seo JS, Seshadri S, Sheehan VA, Sheu WH, Shoemaker MB, Smith NL, Smith JA, Sotoodehnia N, Stilp AM, Tang W, Taylor KD, Telen M, Thornton TA, Tracy RP, Van Den Berg DJ, Vasan RS, Viaud-Martinez KA, Vrieze S, Weeks DE, Weir BS, Weiss ST, Weng LC, Willer CJ, Zhang Y, Zhao X, Arnett DK, Ashley-Koch AE, Barnes KC, Boerwinkle E, Gabriel S, Gibbs R, Rice KM, Rich SS, Silverman EK, Qasba P, Gan W, Consortium NT-OfPM, Papanicolaou GJ, Nickerson DA, Browning SR, Zody MC, Zollner S, Wilson JG, Cupples LA, Laurie CC, Jaquish CE, Hernandez RD, O'Connor TD, Abecasis GR. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. Nature 2021;590:290-299. Epub 2021/02/12
14. Scott EM, Halees A, Itan Y, Spencer EG, He Y, Azab MA, Gabriel SB, Belkadi A, Boisson B, Abel L, Clark AG, Greater Middle East Variome C, Alkuraya FS, Casanova JL, Gleeson JG. Characterization of Greater Middle Eastern genetic variation for enhanced disease gene discovery. Nat Genet 2016;48:1071-1076. Epub 2016/07/19
15. Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Sellars E, Nezhadi SH, Amini A, Arzhangi S, Jalalvand K, Jamali P, Mohammadi Z, Davarnia B, Nikuei P, Oladnabi M, Mohammadzadeh A, Zohrehvand E, Nejatizadeh A, Shekari M, Bagherzadeh M, Shamsi-Gooshki E, Borno S, Timmermann B, Haghdoost A, Najafipour R, Khorram Khorshid HR, Kahrizi K, Malekzadeh R, Akbari MR, Najmabadi H. Iranome: A catalog of genomic variations in the Iranian population. Hum Mutat 2019;40:1968-1984. Epub 2019/07/26
16. GenomeAsia KC. The GenomeAsia 100K Project enables genetic discoveries across Asia. Nature 2019;576:106-111. Epub 2019/12/06
17. Tadaka S, Saigusa D, Motoike IN, Inoue J, Aoki Y, Shirota M, Koshiba S, Yamamoto M, Kinoshita K. jMorp: Japanese Multi Omics Reference Panel. Nucleic Acids Res 2018;46:D551-D557. Epub 2017/10/27
18. Naslavsky MS, Yamamoto GL, de Almeida TF, Ezquina SAM, Sunaga DY, Pho N, Bozoklian D, Sandberg TOM, Brito LA, Lazar M, Bernardo DV, Amaro E, Jr., Duarte YAO, Lebrao ML, Passos-Bueno MR, Zatz M. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. Hum Mutat 2017;38:751-763. Epub 2017/03/24

19. Lim ET, Wurtz P, Havulinna AS, Palta P, Tukiainen T, Rehnstrom K, Esko T, Magi R, Inouye M, Lappalainen T, Chan Y, Salem RM, Lek M, Flannick J, Sim X, Manning A, Ladenvall C, Bumpstead S, Hamalainen E, Aalto K, Maksimow M, Salmi M, Blankenberg S, Ardissino D, Shah S, Horne B, McPherson R, Hovingh GK, Reilly MP, Watkins H, Goel A, Farrall M, Girelli D, Reiner AP, Stitziel NO, Kathiresan S, Gabriel S, Barrett JC, Lehtimaki T, Laakso M, Groop L, Kaprio J, Perola M, McCarthy MI, Boehnke M, Altshuler DM, Lindgren CM, Hirschhorn JN, Metspalu A, Freimer NB, Zeller T, Jalkanen S, Koskinen S, Raitakari O, Durbin R, MacArthur DG, Salomaa V, Ripatti S, Daly MJ, Palotie A, Sequencing Initiative Suomi P. Distribution and medical impact of loss-of-function variants in the Finnish founder population. PLoS Genet 2014;10:e1004494. Epub 2014/08/01

20. Gudbjartsson DF, Helgason H, Gudjonsson SA, Zink F, Oddson A, Gylfason A, Besenbacher S, Magnusson G, Halldorsson BV, Hjartarson E, Sigurdsson GT, Stacey SN, Frigge ML, Holm H, Saemundsdottir J, Helgadottir HT, Johannsdottir H, Sigfusson G, Thorgeirsson G, Sverrisson JT, Gretarsdottir S, Walters GB, Rafnar T, Thjodleifsson B, Bjornsson ES, Olafsson S, Thorarinsdottir H, Steingrimsdottir T, Gudmundsdottir TS, Theodors A, Jonasson JG, Sigurdsson A, Bjornsdottir G, Jonsson JJ, Thorarensen O, Ludvigsson P, Gudbjartsson H, Eyjolfsson GI, Sigurdardottir O, Olafsson I, Arnar DO, Magnusson OT, Kong A, Masson G, Thorsteinsdottir U, Helgason A, Sulem P, Stefansson K. Large-scale whole-genome sequencing of the Icelandic population. Nat Genet 2015;47:435-444. Epub 2015/03/26

21. Maffucci P, Bigio B, Rapaport F, Cobat A, Borghesi A, Lopez M, Patin E, Bolze A, Shang L, Bendavid M, Scott EM, Stenson PD, Cunningham-Rundles C, Cooper DN, Gleeson JG, Fellay J, Quintana-Murci L, Casanova JL, Abel L, Boisson B, Itan Y. Blacklisting variants common in private cohorts but not in public databases optimizes human exome analysis. Proc Natl Acad Sci U S A 2019;116:950-959. Epub 2018/12/29

22. Shirts BH, Pritchard CC, Walsh T. Family-Specific Variants and the Limits of Human Genetics. Trends Mol Med 2016;22:925-934. Epub 2016/10/16

23. He L, Pang X, Chen P, Wang X, Yang T, Wu H. Carrier re-sequencing reveals rare but benign variants in recessive deafness genes. Sci Rep 2017;7:11355. Epub 2017/09/14

24. Slatkin M. Allele age and a test for selection on rare alleles. Philos Trans R Soc Lond B Biol Sci 2000;355:1663-1668. Epub 2000/12/29

25. Raychaudhuri S. Mapping rare and common causal alleles for complex human diseases. Cell 2011;147:57-69. Epub 2011/10/04

26. Niroula A, Vihinen M. How good are pathogenicity predictors in detecting benign variants? PLoS Comput Biol 2019;15:e1006481. Epub 2019/02/12

27. Blekhman R, Man O, Herrmann L, Boyko AR, Indap A, Kosiol C, Bustamante CD, Teshima KM, Przeworski M. Natural selection on genes that underlie human disease susceptibility. Curr Biol 2008;18:883-889. Epub 2008/06/24

28. Bittles A. Consanguinity and its relevance to clinical genetics. Clin Genet 2001;60:89-98. Epub 2001/09/13

29. Tuncbilek E, Koc I. Consanguineous marriage in Turkey and its impact on fertility and mortality. Ann Hum Genet 1994;58:321-329. Epub 1994/10/01

30. Saadat M, Ansari-Lari M, Farhud DD. Consanguineous marriage in Iran. Ann Hum Biol 2004;31:263-269. Epub 2004/06/19

31. Afzal M, Ali SM, Siyal HB. Consanguineous marriages in Pakistan. Pak Dev Rev 1994;33:663-674. Epub 1994/01/01

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2022-12-11

J Clin Res Pediatr Endocrinol 2022;14(3):302-307

# Has the Frequency of Precocious Puberty and Rapidly Progressive Early Puberty Increased in Girls During the COVID-19 Pandemic?

**Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Korcan Demir, Ayhan Abacı, Ece Böber**

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

**What is already known on this topic?**

The timing of puberty is under the control of several factors, such as environmental and nutritional factors, endocrine disruptors, sleep, stress, and bone maturation. However, which one of these factors is the main determinant is not known.

**What this study adds?**

We found that the incidence of newly diagnosed central precocious puberty and rapidly progressive early puberty cases increased during the Coronavirus disease-2019 pandemic period.

## Abstract

**Objective:** Early puberty is development of secondary sex characteristics earlier than the expected normal age range. We subjectively observed an increased frequency of early puberty during the Coronavirus disease-2019 (COVID-19) lockdown and aimed to show the clinical, demographic characteristics of the cases and the change in its incidence.

**Methods:** Female patients with central precocious puberty (CPP, n = 28) and rapidly progressive early puberty (RPEP, n = 61), presenting to our clinic before (January 2019-March 2020) and during the COVID-19 pandemic (April 2020-June 2021) were included.

**Results:** Among 28 CPP cases, six (21%) presented before the pandemic lockdown, whereas 22 (79%) were diagnosed during the COVID-19 pandemic lockdown. While RPEP was seen in 16 (26%) patients before the pandemic, 45 (74%) patients were diagnosed during the lockdown period. Presentation with menarche was seen in 15 RPEP patients; two (13%) were in the prepandemic period and 13 (87%) were in the lockdown period. Chronological age, bone age, bone age to chronological age ratio, height, weight, and body mass index standard deviation scores of patients with RPEP and CPP were similar between the prepandemic and pandemic period.

**Conclusion:** In this cohort, the frequency of CPP and RPP cases were significantly ($p < 0.001$) increased during the COVID-19 pandemic, possibly due to environmental changes.

**Keywords:** LHRH, physical inactivity, distance education, school closures, quarantine

## Introduction

Puberty starts with the elimination of inhibition of the hypothalamic-pituitary axis as a result of the interaction of complex factors. Activation of the gonadotropin-releasing hormone (GnRH) pulse generator occurs with the interaction of kisspeptin and kisspeptin 1 receptor, synchronized operation of neurokinin-B, glutamate, leptin, and androgens. Inhibitory systems are endogenous opioid peptides, such as dynorphin A, gamma-aminobutyric acid (GABA), and macorin ring finger protein-3 (MKRN3) (1). While various hypothalamic genes including KISS1, KISS1R, MKRN3, DLK1 regulate the rate of puberty, micro RNAs provide epigenetic control of puberty by regulating GnRH gene transcription. The timing of puberty is under the control of several factors such as environmental and nutritional factors, endocrine disruptors, sleep, stress, and bone maturation (2,3).

With the Coronavirus disease-2019 (COVID-19) epidemic in our country, primary school education was kept closed from March 2020 to June 2021, and unlike many other countries, children under the age of 18 were isolated at home for most of this period (4). During this period, what appeared

**Address for Correspondence:** Ece Böber MD, Dokuz Eylül University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Turkey
**Phone:** +90 505 525 27 43 **E-mail:** Ece.bober@deu.edu.tr **ORCID:** orcid.org/0000-0001-8828-0892

**Conflict of interest:** None declared
**Received:** 06.01.2022
**Accepted:** 24.04.2022

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

to be a dramatic increase in the cases of central precocious puberty (CPP) and rapidly progressive early puberty (RPEP) were observed in our clinic. The aim of this study was to assess the frequency of admission, risk factors, clinical, and demographic characteristics of such cases before and after the COVID-19 pandemic.

## Methods

### Patients

Female patients diagnosed with CPP and RPEP who presented to our clinic with complaints of precocious puberty before and after the COVID-19 pandemic [15 months' period before and 15 months' period after the start of COVID-19 pandemic lockdown (01 January 2019-30 March 2020 / 01 April 2020-30 June 2021)] were retrospectively assessed. Patients with central or RPEP were included in the study. CPP was defined when breast development was evident before the age of 8 years with a baseline luteinizing hormone (LH) value of $>0.3$ mIU/mL and/or a stimulated LH value of $>5$ mIU/mL (5,6). RPEP is defined as: (a) when girls developed breasts after 8 years of age which reached Tanner stage 3 or 4 within 3-6 months; (b) when girls before the age of nine have breast development at Tanner stage 3 or 4; (c) if menarche occurred before the age of ten; or (d) if their predicted adult height was below target height and there was a decline in predicted adult height during follow-up (5,7,8).

Cases with peripheral precocious puberty, premature thelarche, premature adrenarche, and male patients were not included.

### Methods

Clinical records of the patients before and after/during the COVID-19 pandemic period were retrospectively collected. The data collected regarding anthropometric characteristics included: age (years); height (measured with a sensitivity of 0.1 cm, using a Harpenden stadiometer for those who could stand); weight [measured using a scale with a sensitivity of 0.1 kg (Seca, Hamburg, Germany) with light clothing, (kg)]; body mass index (BMI) (kg/m$^2$); the respective standard deviation (SD) scores (calculated with an online calculator for pediatric endocrinology according to Turkish standards) (9); and pubertal staging evaluated according to the Tanner method (5). Overweight and obesity was defined when the BMI was $\geq 85^{th}$-$<95^{th}$ percentile and and $\geq 95^{th}$ percentile for age and gender, respectively. Bone ages were determined using the Greulich-Pyle radiographic atlas (10). Serum levels of LH (mIU/mL), follicle-stimulating hormone (FSH) (mIU/mL), and estradiol (pg/mL) were measured by immunochemiluminescence (ICMA, ADVIA Centaur XPT, Siemens, USA) immunoassay system. Samples for FSH and LH during GnRH stimulation were obtained 20, 40, and 60 minutes after intravenous administration of 100 µg/m$^2$ (maximum 100 µg) LHRH (LHRH Ferring ampule) when basal FSH and LH levels were not conclusive. In this study, all girls with CPP under the age of eight and girls with RPEP over the age of eight with neurological complaints were evaluated with magnetic resonance imaging (MRI).

### Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences application for Windows, version 21.0 (IBM Inc., Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests revealed that the data did not comply with normal distribution. Descriptive results are presented as median (interquartile range). Comparisons among groups before pandemic and during the pandemic lockdown were made using the Mann-Whitney U test for numeric variables and $x^2$-test for the categorical variables. A p value of $<0.05$ was considered statistically significant.

Institutional approval was granted by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (ethics approval number: 2021/21-05). When applicable, both patients and parents signed the informed consent form to participate in the study.

## Results

A total of 89 cases were included, of which 28 had CPP and 61 had RPEP. Of the 28 cases with CPP, six (21%) were diagnosed during the pre-pandemic period and 22 (79%) were diagnosed during the pandemic period. Of the 61 cases with RPEP, 16 (26%) were diagnosed before the pandemic, while 45 (74%) were diagnosed during the COVID-19 pandemic period. CPP and RPEP female cases in total were approximately three times higher during the COVID-19 pandemic period. Among 15 RPEP cases presenting with early menarche, 13 patients (87%) were diagnosed during the pandemic period.

In total, 20 with CPP and 40 with RPEP were treated with GnRH analogs. Treatment was initiated in 85% (17/20) of the cases diagnosed with CPP, and in 67% (27/40) of cases diagnosed with RPEP during the pandemic period. The height, weight, and BMI SD score values and BMI percentile of CPP and RPEP cases were similar in the prepandemic and pandemic periods (Table 1). Twenty-five percent (7/28) of the cases diagnosed with CPP and 42% (26/61) of the cases diagnosed with RPEP were obese or overweight. Two

of the three obese patients with CPP and 10 of the 13 obese patients with RPP were diagnosed during the pandemic period (Table 2).

In patients with CPP 79% (n = 22) exhibited breast development at Tanner stage 2 and in 21% (n = 6) it was Tanner stage 3. Pubic hair was detected in 42% (n = 12) of the cases diagnosed with CPP. In patients with RPEP 47% (n = 29) exhibited Tanner stage 2 breast development, 40% (n = 24) were at stage 3, and 13% (n = 8) were at Tanner stage 4. GnRH stimulation test was performed in 13 of the CPP patients, 12 of whom were during the pandemic period, and the mean peak LH/FSH average 0.9 (± 0.5) and the mean peak LH was 13 (± 10.6) IU/L.

In total 27 (30%) girls underwent brain MRI. Of these 27 patients, 21 (77%) exhibited no alterations in the hypothalamus-pituitary area of the brain, four (13%) had incidental findings with hypothalamus-pituitary lesions, including two non-functioning pituitary microadenomas and two pineal gland cysts. None of the participants exhibited other hormonal abnormalities. All patients with brain lesions continue to be followed up regularly.

## Discussion

In this study, the frequency of cases with CPP and RPEP increased approximately three times in the pandemic lockdown period compared with the same duration pre-pandemic. Reports from different centers have shown that the incidence of precocious puberty, early menarche, and RPEP in girls has increased after the COVID-19 pandemic (11,12). The exact mechanism of the increase in precocious puberty after restrictions imposed during the pandemic is unknown, and several possible mechanisms have been suggested. One of the mechanisms that may be related to increase in frequency of CPP and RPEP cases during COVID-19 pandemic is the presence of angiotensin-converting enzyme-2 receptor, to which Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) virus binds in the cranial nerves system especially around the olfactory bulb, and the concentration of GnRH neurons and GABAergic neurons in this region. An increased volume of the olfactory bulb has been strongly associated with precocious puberty (13). SARS-CoV-2 may have initiated puberty by disrupting the blood-brain barrier or by direct interaction with neural pathways. In addition, N-methyl-D-aspartate (NMDA) receptors stimulated by inflammatory cytokines may be responsible for increasing GnRH secretion (14,15). However, our patients did not have any sign or history of SARS-CoV-2 virus infection. It should be remembered that, since COVID-19 infection in children is generally considered asymptomatic, patients may not have recognized the related symptoms (16).

**Table 1. Clinical and demographic characteristics of cases diagnosed with early puberty before and after the COVID-19 pandemic**

| | Central puberty precocious (n = 28) | | | Rapidly progressive puberty (n = 61) | | |
|---|---|---|---|---|---|---|
| | Before pandemic (n = 6) | After pandemic (n = 22) | p | Before pandemic (n = 16) | After pandemic (n = 45) | p |
| **Age (years)** | 7.5 (1.0) | 7.5 (0.9) | 0.53 | 8.7 (1.2) | 8.9 (1.1) | 0.58 |
| **Bone age** | 8.2 (1.6) | 8.8 (1.6) | 0.71 | 11 (1.5) | 10.5 (2.1) | 0.74 |
| **Bone age to chronological age ratio** | 1.2 (0.3) | 1.1 (0.2) | 0.93 | 1.1 (0.2) | 1.1 (0.1) | 0.51 |
| **Weight SD score** | 0.7 (1.7) | 0.9 (1.2) | 0.51 | 1.2 (2.0) | 1.0 (1.5) | 0.96 |
| **Height SD score** | 1.5 (2.4) | 1.2 (1.2) | 0.86 | 0.9 (1.8) | 1.4 (1.9) | 0.74 |
| **BMI SD score** | 0.5 (1.9) | 0.5 (0.8) | 0.88 | 1 (1.8) | 0.7 (1.2) | 0.73 |
| **FSH (mIU/mL)** | 3.3 (3.5) | 3.7 (3.2) | 0.53 | 4 (4.1) | 4.5 (3.3) | 0.95 |
| **LH (mIU/mL)** | 0.7 (0.7) | 0.5 (0.9) | 0.84 | 0.9 (1.4) | 0.6 (1.9) | 0.61 |
| **Estradiol (pg/mL)** | 19 (48) | 18 (24) | 0.82 | 27.5 (30) | 24 (24) | 0.73 |

*Data are presented as median (IQR).
IQR: interquartile range, SD: standard deviation, FSH: follicle-stimulating hormone, LH: luteinizing hormone, BMI: body mass index, COVID-19: Coronavirus disease-2019

**Table 2. Frequency of cases**

| | Before pandemic | After pandemic | p |
|---|---|---|---|
| **Central puberty precocious, n (%)** | 6 (21) | 22 (79) | 0.02 |
| **Rapidly progressive early puberty, n (%)** | 16 (26) | 45 (74) | < 0.001 |
| **CPP and RPEP cases, n (%)** | 22 (25) | 67 (75) | < 0.001 |
| **GnRH treatment, n (%)** | 16 (26) | 44 (74) | < 0.001 |
| **Obesity and overweight, n (%)** | 10 (31) | 23 (69) | 0.024 |

GnRH: gonadotropin-releasing hormone, CPP: central precocious puberty, RPEP: rapidly progressive early puberty

COVID-19 lockdown has created chronic and prolonged stress, due to fear of infection and prolonged home quarantine (17). This stress could have increased the release of GnRH through some neurotransmitters and neurons. Studies have shown that prolonged stress accelerates puberty through NMDA, GRF1, CRF, and GABA A receptors in a rat model, and increased cortisol and catecholamines in the mouse model, indirectly (11,12). However, to the best of our knowledge, these mechanisms have not been accepted for humans to date and thus this mechanism remains hypothetical. Another study of the frequency of early puberty in the use of methylphenidate showed that dopamine and norepinephrine may trigger puberty, through transporter blockage, as their concentrations in synaptic gaps increase (18).

We did not include any assessment of patient stress levels during the COVID-19 outbreak and the number of quarantine days. However, several reports have documented both increased parental stress and children's psychological problems during the pandemic, using measurable parameters (19,20). However, at present the evidence for this is very limited.

In the present study, it was found that 28% of CPP and 69% of RPEP cases were either obese or overweight and who were diagnosed during the pandemic period. Nutrition is the most important determinant of pubertal maturation. It is well-known that puberty may be triggered by nutritional correction in malnourished, adopted children (21,22). It has been shown that the prevalence of obesity increased during the COVID-19 pandemic in the USA, especially between the ages of 5-9 years (23). Furthermore, overnutrition in low-birth-weight girls may lead to accelerated puberty, ovarian dysfunction, and polycystic ovary syndrome in the future, by various factors and mechanisms, including DNA methylation, microRNAs, and microbiota (22). There were no low-birth-weight cases among our patients.

It is also well established that vigorous physical activity delays the onset of puberty (24). There is no study investigating the mechanisms by which limited physical activity may affect pubertal timing or sedentary lifestyles will likely accelerate the onset of puberty. In epidemiological studies of adolescent girls in India and Ethiopia, the age of menarche was earlier in sedentary urban girls compared to girls in rural areas with increased daily physical activity (25,26).

In many studies evaluating the relationship between exposure to endocrine disruptors and the timing of puberty in girls, many endocrine disruptor chemicals have been associated with early pubertal changes, although the effects vary according to exposure time, period, and the gender of the patient (27,28). We suggest that exposure to endocrine disruptors probably increased through more intensive use of disinfectants and the use of immune-boosting supplements in the COVID-19 pandemic.

Distance education and the use of electronic devices and screen exposure have increased in Turkey during the COVID-19 pandemic. A study by Stagi et al. (11) showed that the total screen time children were exposed to has increased 2.5 times during the COVID-19 pandemic. High melatonin levels detected at night in prepubertal children were found to decrease with puberty (29). Salti et al. (30) showed a decrease in urinary melatonin concentration was associated with children's television screen exposure. It is thought that increased use of electronic devices may lead to a decrease in melatonin levels, causing the onset of pubertal development. The study of Tsai et al. (31) showed that internet use in adolescents was positively correlated with early puberty frequency. These authors estimated that increased use of electronic devices increased obesity and increased BMI may induce early puberty (31). Given these findings, we also suggest that prolonged exposure of children to the screen of electronic devices, such as televisions and computers, amy also be a factor in the increase in the frquency of cases with CPP and RPEP during the COVID-19 pandemic.

### Study Limitations

COVID-19 infection and endocrine effects have not been explored previously. Our study is one of the first studies to examine this, with regard to the interaction between the lockdown period and the endocrine system. We also believe that our study may provide further information about the specific mechanisms initiating puberty. The main limitation of this study was the short follow-up period of our patients, due to the closure of the outpatient clinics in lockdown period for a long time, during which only emergent cases were examined. The other limitation of this study was that, although there are a number of hypothetical factors which may be associated with the increased frequency of CPP and RPEP, in this study evidence of past SARS-CoV-2 infection, patient screen time, sleep time, physical activity time, dietary changes, and additional stress factors were not investigated

## Conclusion

In conclusion, the frequency and number of CPP and RPEP cases increased during the COVID-19 pandemic compared with the same 15 month period prior to the pandemic. Several factors which have increased during the restrictions imposed in an attempt to control infections rates, such as changes in dietary habits, greater use of technology

products, decrease in physical activity, and increase in sedentary lifestyle because of distance education and quarantine, could precipitate an increase in numbers of cases of precocious puberty. Further larger studies are needed to investigate the possible causes of the association between the COVID-19 pandemic and precocious puberty.

### Acknowledgement

The authors are grateful to the patients and their parents for participating in this study.

### Ethics

**Ethics Committee Approval:** Approval was granted by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (approval number: 2021/21-05, date: 14.07.2021).

**Informed Consent:** All participants provided informed consent.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Korcan Demir, Ayhan Abacı, Ece Böber, Concept: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Korcan Demir, Ayhan Abacı, Ece Böber, Design: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Korcan Demir, Ayhan Abacı, Ece Böber, Data Collection or Processing: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Analysis or Interpretation: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Literature Search: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci, Writing: Kübra Yüksek Acinikli, İbrahim Mert Erbaş, Özge Besci.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Livadas S, Chrousos GP. Molecular and Environmental Mechanisms Regulating Puberty Initiation: An Integrated Approach. Front Endocrinol (Lausanne) 2019;10:828.
2. Plant TM. Neuroendocrine control of the onset of puberty. Front Neuroendocrinol 2015;38:73-88. Epub 2015 Apr 22
3. Aguirre RS, Eugster EA. Central precocious puberty: From genetics to treatment. Best Pract Res Clin Endocrinol Metab 2018;32:343-354.
4. https://en.wikipedia.org/wiki/Timeline_of_the_COVID-19_pandemic_in_Turkey (Accessed: December 9th, 2021).
5. Cheuiche AV, da Silveira LG, de Paula LCP, Lucena IRS, Silveiro SP. Diagnosis and management of precocious sexual maturation: an updated review. Eur J Pediatr 2021;180:3073-3087. Epub 2021 Mar 21
6. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. J Clin Endocrinol Metab 2007;92:1424-1429. Epub 2007 Feb 6
7. Berberoğlu M. Precocious puberty and normal variant puberty: definition, etiology, diagnosis and current management. J Clin Res Pediatr Endocrinol 2009;1:164-174. Epub 2009 May 2
8. Bereket A. A Critical Appraisal of the Effect of Gonadotropin-Releasing Hormon Analog Treatment on Adult Height of Girls with Central Precocious Puberty. J Clin Res Pediatr Endocrinol 2017;9(Suppl 2):33-48.
9. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol 2015;7:280-293.
10. Radiographic atlas of skeletal development of the hand and wrist. Calif Med 1950;73:378.
11. Stagi S, De Masi S, Bencini E, Losi S, Paci S, Parpagnoli M, Ricci F, Ciofi D, Azzari C. Increased incidence of precocious and accelerated puberty in females during and after the Italian lockdown for the coronavirus 2019 (COVID-19) pandemic. Ital J Pediatr 2020;46:165.
12. Street ME, Sartori C, Catellani C, Righi B. Precocious Puberty and Covid-19 Into Perspective: Potential Increased Frequency, Possible Causes, and a Potential Emergency to Be Addressed. Front Pediatr 2021;9:734899.
13. Karaoglan M, Çolakoğlu Er H. The relationship between the olfactory bulb and precocious puberty: from the nose to the pituitary. J Pediatr Endocrinol Metab 2019;32:1013-1021.
14. Hori H, Kim Y. Inflammation and post-traumatic stress disorder. Psychiatry Clin Neurosci 2019;73143-73153. Epub 2019 Feb 21
15. Adams MM, Flagg RA, Gore AC. Perinatal changes in hypothalamic N-methyl-D-aspartate receptors and their relationship to gonadotropin-releasing hormone neurons. Endocrinology 1999;140:2288-2296.
16. Zimmermann P, Curtis N. Coronavirus Infections in Children Including COVID-19: An Overview of the Epidemiology, Clinical Features, Diagnosis, Treatment and Prevention Options in Children. Pediatr Infect Dis J 2020;39:355-368.
17. Singh S, Roy D, Sinha K, Parveen S, Sharma G, Joshi G. Impact of COVID-19 and lockdown on mental health of children and adolescents: A narrative review with recommendations. Psychiatry Res 2020;293:113429. Epub 2020 Aug 24
18. Ergür AT, Gül H, Gül A. Methylphenidate and Central Precocious Puberty: A Probable Side Effect among Seven Children with the Attention Deficit Hyperactivity Disorder. Clin Psychopharmacol Neurosci 2019;17:446-449.
19. Spinelli M, Lionetti F, Pastore M, Fasolo M. Parents' Stress and Children's Psychological Problems in Families Facing the COVID-19 Outbreak in Italy. Front Psychol 2020;11:1713.
20. Brown SM, Doom JR, Lechuga-Peña S, Watamura SE, Koppels T. Stress and parenting during the global COVID-19 pandemic. Child Abuse Negl 2020;110:104699. Epub 2020 Aug 20
21. Virdis R, Street ME, Zampolli M, Radetti G, Pezzini B, Benelli M, Ghizzoni L, Volta C. Precocious puberty in girls adopted from developing countries. Arch Dis Child 1998;78:152-154.
22. Proos L, Gustafsson J. Is early puberty triggered by catch-up growth following undernutrition? Int J Environ Res Public Health 2012;9:1791-1809. Epub 2012 May 9
23. Jenssen BP, Kelly MK, Powell M, Bouchelle Z, Mayne SL, Fiks AG. COVID-19 and Changes in Child Obesity. Pediatrics 2021;147:2021050123. Epub 2021 Mar 2
24. Weiss Kelly AK, Hecht S; Council on Sports Medicine and Fitness. The Female Athlete Triad. Pediatrics 2016;138:20160922. Epub 2016 Jul 18

25. Choudhary S, Khichar S, Dabi D, Parakh M, Dara PK, Parakh P, Vyas S, Deopa B. Urban Rural Comparison of Anthropometry and Menarcheal Status of Adolescent School Going Girls of Jodhpur, Rajasthan, India. J Clin Diagn Res 2016;10:8-12. Epub 2016 Oct 1

26. Ayele E, Berhan Y. Age at menarche among in-school adolescents in Sawla Town, South Ethiopia. Ethiop J Health Sci 2013;23:189-200.

27. Abaci A, Demir K, Bober E, Buyukgebiz A. Endocrine disrupters - with special emphasis on sexual development. Pediatr Endocrinol Rev 2009;6:464-475.

28. Wolff MS, Pajak A, Pinney SM, Windham GC, Galvez M, Rybak M, Silva MJ, Ye X, Calafat AM, Kushi LH, Biro FM, Teitelbaum SL; Breast Cancer and Environment Research Program. Associations of urinary phthalate and phenol biomarkers with menarche in a multiethnic cohort of young girls. Reprod Toxicol 2017;67:56-64. Epub 2016 Nov 13

29. Waldhauser F, Weiszenbacher G, Tatzer E, Gisinger B, Waldhauser M, Schemper M, Frisch H. Alterations in nocturnal serum melatonin levels in humans with growth and aging. J Clin Endocrinol Metab 1988;66:648-652.

30. Salti R, Tarquini R, Stagi S, Perfetto F, Cornélissen G, Laffi G, Mazzoccoli G, Halberg F. Age-dependent association of exposure to television screen with children's urinary melatonin excretion? Neuro Endocrinol Lett 2006;27:73-80.

31. Tsai MC, Strong C, Chen WT, Lee CT, Lin CY. Longitudinal impacts of pubertal timing and weight status on adolescent Internet use: Analysis from a cohort study of Taiwanese youths. PLoS One 2018;13:0197860.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2021-11-9

J Clin Res Pediatr Endocrinol 2022;14(3):308-312

# Autoimmune Primary Adrenal Insufficiency in Children

**Nádia Mourinho Bala[1], Raquel S. Gonçalves[2], Joana Serra Caetano[3], Rita Cardoso[3], Isabel Dinis[3], Alice Mirante[3]**

*[1]Hospital Beatriz Ângelo, Department of Endocrinology, Loures, Portugal*
*[2]Pediatric Hospital of Coimbra, Department of Pediatrics, Coimbra, Portugal*
*[3]Pediatric Hospital of Coimbra, Department of Pediatric Endocrinology, Diabetes and Growth, Coimbra, Portugal*

**What is already known on this topic?**

Autoimmune adrenal insufficiency is a rare condition in pediatric age groups. The symptoms at presentation are frequently non-specific and this disease is often misdiagnosed. In order to avoid adverse outcomes, a prompt diagnosis should be established and the treatment initiated immediately.

**What this study adds?**

This study describes the demographic characteristics, clinical presentation and laboratorial findings of autoimmune adrenal insufficiency in the pediatric age group in one portuguese reference center. Additionally, by increasing pediatricians' awareness of this potential life-threatening disease, we hope to contribute to an earlier diagnosis.

## Abstract

**Objective:** Primary adrenal insufficiency (PAI) is a rare condition in children, and is potentially life-threatening. The most common cause is congenital adrenal hyperplasia, and autoimmune etiology is the most frequent acquired cause in this age group. Symptoms are usually non-specific and, when suspected, investigation should include adrenocorticotropin hormone (ACTH) and morning serum cortisol measurement and, in some cases, a cosyntropin test to confirm the diagnosis. Prompt treatment is essential to prevent an adverse outcome.

**Methods:** We retrospectively collected clinical and laboratory data from adrenal insufficiency due to autoimmune adrenalitis, observed from 2015 to 2020 in a pediatric endocrinology department of a tertiary care hospital.

**Results:** Eight patients were identified, seven males and one female, with age at diagnosis between 14 and 17 years. The symptoms at presentation ranged from non-specific symptoms, such as chronic fatigue and weight loss, to a severe presentation, with altered mental status and seizures. The median duration of symptoms was 4.5 months. The diagnosis was confirmed by serum cortisol and plasma ACTH measurement and all were confirmed to have autoimmune etiology (positive anti-adrenal antibodies). At diagnosis, the most common laboratory abnormality was hyponatremia. All patients were treated with hydrocortisone and fludrocortisone. One patient presented with evidence of type 2 autoimmune polyglandular syndrome.

**Conclusion:** PAI is a rare condition in the pediatric age group. Due to non-specific symptoms, a high index of suspicion is necessary to establish a prompt diagnosis. Once an autoimmune etiology is confirmed, it is important to initiate the appropriate treatment and search for signs and symptoms of other autoimmune diseases during follow-up.

**Keywords:** Primary adrenal insufficiency, pediatric adrenal insufficiency, Addison's disease

## Introduction

Adrenal insufficiency is a rare condition caused by the dysfunction of the adrenal cortex, resulting in impaired secretion of glucocorticoids and/or mineralocorticoids (1,2). These hormones play an important role in energy, salt and fluid homeostasis. Thus, adrenal insufficiency is a potentially life-threatening condition (2). It comprises a heterogenous group of both congenital and acquired disorders with autoimmune adrenalitis being the most common cause of acquired adrenal insufficiency (1).

**Address for Correspondence:** Nádia Mourinho Bala MD, Hospital Beatriz Ângelo, Department of Endocrinology, Loures, Portugal
**Phone:** +00351219847200 **E-mail:** nadia.mourinho@gmail.com **ORCID:** orcid.org/0000-0002-5834-4848

**Conflict of interest:** None declared
**Received:** 10.02.2022
**Accepted:** 09.05.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

In children, the diagnosis of adrenal insufficiency can be very challenging. Patients with adrenal crisis generally present with characteristic features of acute dehydration, hypotension, abdominal pain, vomiting and/or altered mental status. However, the features of chronic adrenal insufficiency are vague and non-specific, such as chronic fatigue, anorexia, nausea, vomiting, loss of appetite, weight loss and recurring abdominal pain. These symptoms may mimic gastrointestinal or psychiatric disorders. Hyperpigmentation of skin and mucosal tissue should raise the index of suspicion for primary adrenal insufficiency (PAI) (3,4,5,6). Classic laboratory findings include hyponatremia, hyperkalemia, hypoglycemia, and metabolic acidosis (1,6).

In PAI, cortisol deficiency leads to an activation of the hypothalamic-pituitary axis with a subsequent increase of plasma adrenocorticotropic hormone (ACTH), which enhances stimulation of the adrenal cortex. Due to the disruption of adrenal mineralocorticoid synthesis, there is an increase in renin release by the juxtaglomerular cells of the kidneys (2). Thus, screening tests should include morning cortisol and ACTH levels and plasma renin activity (1,6). Once diagnosis is made, treatment intends to mimic normal cortisol secretion, with replacement of glucocorticoid and mineralocorticoid according to body surface area (7).

The aim of the present study was to describe clinical presentation, biochemical abnormalities, and treatment in patients with autoimmune adrenal insufficiency followed in a department of pediatric endocrinology of a tertiary care hospital.

## Methods

This is a retrospective and descriptive study of pediatric patients with the diagnosis of autoimmune PAI (APAI), from January 2015 to December 2020, followed at a single pediatric endocrinology unit of a tertiary hospital in Portugal. The diagnosis of APAI was based on ACTH elevation (> 2 times upper limit), low serum cortisol (< 5 ug/dL) and positive anti-adrenal antibodies. The anti-adrenal antibodies were measured by indirect immunofluorescence assay, using as a substract the primate adrenal cortex. The initial dilution was 1:4. Data was collected from medical records and included demographic characterization, personal and familial history, age of onset of symptoms, clinical and laboratory findings, treatment and evolution. Both the data collection and the analysis were performed were anonymized, respecting patient privacy and ethical considerations. All laboratory testing was performed in our institutional laboratory and the reference ranges are: ACTH 7.6-63 pg/mL; active renin concentration 7-76 uU/mL; aldosterone 40-310 pg/mL; serum Na+ 136-146 mmol/L; and serum K+ 3.5-5.1 mmol/L.

### Statistical Analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). Continuous data are described as median and interquartile range and categorical data are described as the number of cases (%).

## Results

Eight cases were identified (seven males and one female) with median age at diagnosis of 15.1 years (Q1;Q3 13.4;16.1). Five patients had at least one first degree relative with autoimmune disease (AID), namely systemic lupus erythematosus, type 1 diabetes mellitus, Graves disease and APAI. One patient had a second-degree family history of type 1 diabetes mellitus and Graves disease. The most frequent symptoms at presentation were anorexia (n = 5), weight loss (n = 5), chronic fatigue (n = 5), nausea and vomiting (n = 4), hyperpigmentation (n = 3), abdominal pain (n = 2) and myalgia (n = 2). Less frequent symptoms included altered mental status and seizures due to severe hyponatremia (Na+ 113 mmol/L), reported in one case, and salt craving reported in another patient (Table 1). The median duration of symptoms before diagnosis was 4.5 months. On observation, three patients had hypotension and two were febrile. In laboratory evaluation, seven patients presented with hyponatremia (median Na+ 122.5 mmol/L, Q1;Q3 118;126.5), five patients with hyperkalemia (median K+ 5.3 mmol/L, Q1;Q3 5.2;5.5), three patients metabolic acidosis (median pH 7.22) and two patients presented with hypoglycemia (52 mg/dL and 63 mg/dL). At presentation, median serum cortisol was 1.35 µg/dL (Q1;Q3 1;2.2) and ACTH was 1250 pg/mL (Q1;Q3 1040;1250). Seven patients presented with low aldosterone level (median 34.7 pg/mL) and six patients presented with high active renin concentration (median 8180 uU/mL) (Table 2). All patients had strong positive anti-adrenal antibodies, by immunofluorescence assay. Six of these patients had presented to a health service at least once before, with the same symptoms, and were misdiagnosed. The most common diagnosis in these cases was acute gastroenteritis. One patient was admitted one month before the diagnosis due to weight loss, loss of appetite and fatigue and an eating disorder diagnosis was assumed. In this case, having a pre-existing personal history of anorexia nervosa contributed for the misdiagnosis. Patients were treated with hydrocortisone and fludrocortisone, with a median dosage of 16.7 mg/m$^2$/day and 87.5 mcg/day, respectively. The median time

of follow up was 29 months. During this period, one patient presented with hyperthyroidism in the context of Graves disease, contributing to a diagnosis of polyglandular autoimmune syndrome type 2, and he also had selective IgA deficiency. Another patient presented with increased anti-thyroid peroxidase antibodies, even though he was asymptomatic at the time of writing.

## Discussion

APAI is a rare diagnosis in children. In a study published by Perry et al. (8), including 103 patients, the most common cause of PAI in the pediatric population was attributed to congenital adrenal hyperplasia followed by autoimmune etiology (12.7% of all cases). The cases of APAI associated with autoimmune polyendocrinopathy syndrome type 1 (APS1) were diagnosed at a younger age when compared with non-APS1 patients (10.7 vs 14.6 years-old) (8). In our study the median age at diagnosis was 15.1 years and there were no cases in association with APS1. The diagnosis of APAI was more common in males, as reported in other series (9). So, despite APAI being more frequent in females in the general population, in the pediatric age group it seems to be more frequently diagnosed in males.

The usual presentation in adolescent years and with non-specific symptoms (anorexia, weight loss and chronic fatigue) may lead to misdiagnosis, more severe presentation and delayed treatment. Although less frequent than other diagnoses that may present (depression, anorexia nervosa, etc), organic disease should always be excluded, even in this age group. More specific symptoms, such as hyperpigmentation of the skin and mucous membranes and salt craving, were less common. Hypoglycemia was detected in only two cases, traditionally more frequent in cases of central adrenal insufficiency than in cases of PAI. There was a considerable time gap from first symptoms until the diagnosis, frequently over 4-6 months. Most patients had already consulted a medical doctor prior to the diagnosis due to the same symptoms. However, owing to a lack of specificity of symptoms such as chronic fatigue, loss of appetite, weight loss and nausea and vomiting, frequently gastrointestinal illness was diagnosed. In one

**Table 1. Patient demographic and clinical characteristics in terms of gender, age at diagnosis, presence of familial history of autoimmune diseases, symptoms at presentation and duration before diagnosis**

| ID | Gender | Age at diagnosis (years) | Familial history of AID | Presenting symptoms | Symptoms duration (months) |
|---|---|---|---|---|---|
| **1** | M | 16 | P | Anorexia, astenia, weight loss, back pain, myalgia, hyperpigmentation | 3 |
| **2** | M | 15 | NP | Astenia, weight loss, abdominal pain, vomiting, myalgias, hyperpigmentation | 6 |
| **3** | M | 17 | NP | Astenia, anorexia, weight loss | < 1 |
| **4** | F | 15 | P | Astenia, anorexia, weight loss, vomiting and postural dizziness | 1 |
| **5** | M | 14 | NP | Anorexia, weight loss, vomiting, fever | 6 |
| **6** | M | 12 | P | Hyperpigmentation | 6 |
| **7** | M | 16 | P | Astenia, anorexia, postural dizziness, salt craving | < 1 |
| **8** | M | 15 | P | Seizures, altered mental status, abdominal pain, vomiting | 12 |

M: male, F: female, AID: autoimmune disease, P: present, NP: non present

**Table 2. Clinical and laboratory findings at presentation**

| ID | At presentation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hypotension | Fever | pH | Na + (mmol/L) | K + (mmol/L) | $HCO^{3}$- (mmol/L) | Serum glucose (mg/dL) | Serum creatinine (mg/dL) | ACTH (pg/mL) | Serum cortisol (µg/dL) | Serum active renin concentration (uU/mL) |
| 1 | P | NP | 7.37 | 125 | 5.13 | 23.7 | 88 | 0.95 | > 1250 | < 1.0 | 8785 |
| 2 | NP | NP | NA | 116 | 4.0 | NA | NA | NA | NA | NA | NA |
| 3 | P | NP | 7.38 | 128 | 4.48 | 26.6 | 101 | 0.82 | > 1250 | 2.6 | 9730 |
| 4 | NP | P | 7.35 | 120 | 5.6 | 23.7 | 70 | 0.5 | 1040 | 3.6 | 3505 |
| 5 | P | P | 7.21 | 120 | 5.3 | 24 | 92 | 0.46 | > 2000 | 1.7 | 84 |
| 6 | NP | NP | 7.38 | 141 | 4.04 | 23.7 | 86 | 0.43 | > 1250 | 1.8 | 233 |
| 7 | NP | NP | 7.31 | 125 | 5.4 | 23.7 | 63 | 0.86 | 1241 | 1 | 7575 |
| 8 | NP | NP | 7.22 | 113 | 5.2 | 20 | 52 | 0.66 | 802 | 0.9 | 20840 |

P: present, NP: not present, NA: not available, ACTH: adrenocorticotropin hormone

case, an exacerbation of an eating disorder was assumed to be the diagnosis. Similarly to our findings, Hsieh and White (10) described the case of one patient diagnosed with anorexia nervosa and admitted to the inpatient psychiatry unit before a diagnosis of adrenal insufficiency was reached. It is important to include endocrine entities, namely adrenal insufficiency and hyperthyroidism, in the differential diagnosis of an eating disorder. The presence of symptoms, such as fatigue, weight loss, and upper gastrointestinal distress, which are associated with hyponatremia, should always trigger suspicion of adrenal insufficiency, particularly in patients with positive personal or familial history of AID. Nonetheless, the diagnosis is frequently delayed, resulting in a clinical presentation with acute life-threatening adrenal crisis with hypotension, marked acute abdominal symptoms and marked laboratory abnormalities, requiring immediate treatment. In our series, seven of the eight patients presented with adrenal crisis. The only patient that presented in an early stage of the disease had a familial history of autoimmune adrenal insufficiency, and the hyperpigmentation immediately raised suspicion of this diagnosis. When the diagnosis of PAI is suspected, morning serum cortisol and plasma ACTH should be measured. In most cases, the diagnosis is highly probable when cortisol is $<5$ µg/dL in combination with a plasma ACTH elevated more than 2-fold above the upper limit of the reference interval for the specific assay, in a blood sample collected at 8:00 am (2). In the presence of equivocal values, a cosyntropin test, currently regarded as the diagnostic "gold standard" for the diagnosis of PAI, should be performed (2,6). In our series this test was not necessary, since all patients presented with unequivocally low serum cortisol and high levels of ACTH. The treatment of PAI consists of glucocorticoid and mineralocorticoid replacement (11). The majority of patients presented with mineralocorticoid deficiency at the time of diagnosis. However, even in cases when there is no mineralocorticoid deficiency at presentation, it might eventually occur in the course of the disease. The treatment for children mainly involves the administration of short acting glucocorticoids, particularly hydrocortisone (1). It is important to optimize the doses in order to mimic the physiological circadian rhythm, and to prevent side effects, such as growth suppression, obesity, metabolic syndrome, diabetes and osteoporosis (11). In our series, the patient taking the highest daily hydrocortisone dosage (31.6 mg/m$^2$/day) had a simultaneous diagnosis of Graves disease, a condition that is well-known to accelerate cortisol metabolism. The thyrotoxic state shortens the half-life of cortisol, due to an increased turnover rate, mediated by 11$\beta$-hydroxysteroid dehydrogenase and 5-$\alpha$ reductase enzymes (12). Thus, cortisol requirements are increased, and a higher daily dosage of hydrocortisone is often needed in these cases.

It is recommended to monitor glucocorticoid replacement by clinical assessment in children, using metrics such as growth velocity, weight, blood pressure and energy levels (2). As in other chronic diseases, compliance is a very important factor associated with disease control, and is particularly challenging during adolescence. In the management of adrenal insufficiency, it is extremely important to educate the patients and their families in order to actively increase glucocorticoid dosage when exposed to stress, such as during severe systemic infection or trauma, to minimize the risk of adrenal crisis. When oral hydrocortisone formulations cannot be used, parenteral hydrocortisone should be administered (6). In our patients, not one case of adrenal crisis was registered after diagnosis. Autoimmune adrenalitis is strongly associated with specific HLA haplotypes and with polymorphisms in the gene for cytotoxic T lymphocyte-associated antigen-4, which may be broadly involved in susceptibility to AID (13). About half of adult patients with lymphocytic adrenalitis will also have AID of a different endocrine organ or tissue (14). During the follow-up it is important to be aware of signs and symptoms of other AID, namely thyroid disease, autoimmune gastritis, type 1 diabetes mellitus, celiac disease and premature ovarian failure (2). In our series, one patient presented with type 2 APS that included hyperthyroidism due to Graves disease. The same patient also had IgA deficiency, which has a well-established relationship with AID, more often to type 1 diabetes, autoimmune thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, celiac disease and myasthenia gravis (15). The association between IgA deficiency and autoimmune adrenal insufficiency is very rare (16). Another patient presented with positive anti-peroxidase antibodies but had no clinical disease at the time of writing.

### Study Limitations

Our study has some limitations. Firstly, our study included a small sample size of a single reference center. Secondly, the nature of the study required us to rely on data from medical records.

## Conclusion

In conclusion, APAI is a rare, potentially life-threatening diagnosis in children. A high index of suspicion is required for an accurate diagnosis due to non-specific and insidious symptoms. Once diagnosis is made, an adequate treatment should be promptly provided, and clinical and subclinical manifestations of other autoimmune disorders should be

carefully investigated during follow-up. It is also crucial to educate patients and their families regarding treatment adjustments to stress to avoid adrenal crises over time.

### Ethics

**Ethics Committee Approval and Informed Consent:** The study design was approved by the Ethics Committee of the Hospital and University Center of Coimbra (OBS.SF.60-2021 date: 22.09.2021), and the requirement for written informed consent was waived due to the retrospective nature of the study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Joana Serra Caetano, Rita Cardoso, Isabel Dinis, Alice Mirante, Concept: Alice Mirante, Design: Alice Mirante, Data Collection or Processing: Nádia Mourinho Bala, Raquel S. Gonçalves, Analysis or Interpretation: Nádia Mourinho Bala, Literature Search: Nádia Mourinho Bala, Raquel S. Gonçalves, Writing: Nádia Mourinho Bala, Raquel S. Gonçalves, Joana Serra Caetano, Rita Cardoso, Isabel Dinis.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Eyal O, Levin Y, Oren A, Zung A, Rachmiel M, Landau Z, Schachter-Davidov A, Segev-Becker A, Weintrob N. Adrenal crises in children with adrenal insufficiency: epidemiology and risk factors. Eur J Pediatr 2019;178:731-738. Epub 2019 Feb 26
2. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2016;101:364-389. Epub 2016 Jan 13
3. Park J, Didi M, Blair J. The diagnosis and treatment of adrenal insufficiency during childhood and adolescence. Arch Dis Child 2016;101:860-865.
4. Shulman DI, Palmert MR, Kemp SF; Lawson Wilkins Drug and Therapeutics Committee. Adrenal insufficiency: still a cause of morbidity and death in childhood. Pediatrics 2007;119:484-494. Epub 2007 Jan 22
5. Rushworth RL, Torpy DJ, Stratakis CA, Falhammar H. Adrenal Crises in Children: Perspectives and Research Directions. Horm Res Paediatr 2018;89:341-351. Epub 2018 Jun 6
6. Auron M, Raissouni N. Adrenal insufficiency. Pediatr Rev 2015;36:92-102.
7. Simm PJ, McDonnell CM, Zacharin MR. Primary adrenal insufficiency in childhood and adolescence: advances in diagnosis and management. J Paediatr Child Health 2004;40:596-599.
8. Perry R, Kecha O, Paquette J, Huot C, Van Vliet G, Deal C. Primary adrenal insufficiency in children: twenty years experience at the Sainte-Justine Hospital, Montreal. J Clin Endocrinol Metab 2005;90:3243-3250. Epub 2005 Apr 5
9. Raposo NC, Kazakova E, Pina R, Lopes L. Addison's Disease in Paediatric Age. Rev Port Endocrinol Diabetes Metab 2020;15:151-155.
10. Hsieh S, White PC. Presentation of primary adrenal insufficiency in childhood. J Clin Endocrinol Metab 2011;96:925-928. Epub 2011 Apr 6
11. Güran T. Latest Insights on the Etiology and Management of Primary Adrenal Insufficiency in Children. J Clin Res Pediatr Endocrinol 2017;9(Suppl 2):9-22. Epub 2017 Dec 27
12. Hoshiro M, Ohno Y, Masaki H, Iwase H, Aoki N. Comprehensive study of urinary cortisol metabolites in hyperthyroid and hypothyroid patients. Clin Endocrinol (Oxf) 2006;64:37-45.
13. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Rønningen KS, Guja C, Ionescu-Tirgovişte C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003;423:506-511. Epub 2003 Apr 30
14. Sperling MA. Pediatric Endocrinology. Fourth edition. Philadelphia, PA: Elsevier Health Books. 2014;508-514.
15. Singh K, Chang C, Gershwin ME. IgA deficiency and autoimmunity. Autoimmun Rev 2014;13:163-177. Epub 2013 Oct 21
16. Baleva MP, Mihaylova S, Yankova P, Atanasova I, Nikolova-Vlahova M, Naumova E. A rare case of Addison's disease, hepatitis, thyreoiditis, positive IgG anti-tissue transglutaminase antibodies and partial IgA deficiency. Cent Eur J Immunol 2016;41:217-220. Epub 2016 Jul 15

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2022-1-21

J Clin Res Pediatr Endocrinol 2022;14(3):313-323

# Analysis of Apoptotic, Clinical, and Laboratory Parameters in Type 1 Diabetes and Early Diabetic Nephropathy: Clustering and Potential Groups Evaluation for Additional Therapeutic Interventions

**Ievgeniia Burlaka**

*Bogomolets National Medical University, Department of Pediatrics, Kyiv, Ukraine*

### What is already known on this topic?

Diabetic nephropathy (DN) is marked by pathological changes occurring in the renal glomeruli that lead to the development of albuminuria, hypertension, and progressive decline in renal function. Traditionally, different factors, such as the duration of diabetes, puberty, age at onset, family history of diabetic complications, family history of insulin resistance, type 1 and 2 diabetes, genetic factors, race/ethnicity, modifiable, glycemic (metabolic) control, smoking, hyperlipidemia, intrauterine exposure, obesity, pregnancy, social status discussed in terms of risk factors and predictors of type 1 diabetes (T1D) complications, including DN. However, complex studies dedicated to evaluating the possibility of using clinical markers in combination with markets of apoptosis and hypoxia for T1D and DN stratification in children have not yet been performed.

### What this study adds?

We believe that our results showing that T1D pediatric patients with increased platelets (PLT) count, hyperfiltration and reduced anti-apoptotic defense may be a cornerstone group for therapeutic interventions, i.e. antioxidants along with optimal glycemic control. DN group found to have somewhat increased PLT count, high frequency of diabetic ketoacidosis episodes/year, high microalbuminuria, prominent increase in HIF level, prominent disturbances in apoptosis controlling factors BcL-xL and caspase-3 requires additional therapeutic interventions, i.e. antioxidants, anti-apoptotic effectors along with optimal glycemic control, management of hypertension and albuminuria.

## Abstract

**Objective:** Type 1 diabetes (T1D) is one of the most prevalent chronic illnesses diagnosed in childhood. Diabetic nephropathy (DN) is one of the commonest complication of T1D. Therefore the development of specific treatment that arrests progression of DN based on an individual approach would be beneficial. Analysis of criteria of apoptosis, and clinical, and laboratory characteristics in T1D and early DN in the framework of clustering may be helpful in the identification of potential groups for additional therapeutic interventions.

**Methods:** A survey of 104 children (62 males, 42 females) with T1D and DN aged 2 to 17 years in the Endocrinology unit of Clinical Pediatric Hospital No 6 (Kyiv, Ukraine) was performed. Clinical data (age, gender, disease duration, blood pressure), conventional laboratory markers including complete blood count, serum cholesterol, hemoglobin A1c (Hb1Ac), glomerular filtration rate (GFR), and microalbuminurea (MAU), and markers of apoptosis (BcL-xL, caspase-3) and transcriptional factor HIF-1alfa were analyzed.

**Results:** A cluster group in T1D children was characterized by somewhat higher number of platelets (PLT) - $344.9 \pm 7.88 \cdot 10^9$/L, increased GFR up to hyperfiltration level $124.5 \pm 8.86$ mL/min/1.73 $m^2$ and decreased anti-apoptotic defense - BcL-xL $144.9 \pm 2.35$ a.u. was identified. Children with DN may be divided into three groups based on age, body mass index, systolic blood pressure, PLT count, erthyrocyte sedimentation rate, albumin/globulin ratio, serum cholesterol, Hb1Ac, number of diabetic ketoacidosis (DKA) episodes, GFR, MAU, HIF-1alfa, Bcl-xL, caspase-3 levels. Among children with early DN a cluster characterized by the following parameters was found: PLT count - $311. \pm 12.05 \cdot 10^9$/L, frequency of DKA episodes - $4.82 \pm 0.26$ episodes/year, MAU - $112.0 \pm 10.12$ mm/24 h, HIF - $200.5 \pm 3.49$ a.u., BcL-xL - $128.8 \pm 3.1$ a.u., and caspase-3 - $159.6 \pm 5.5$ a.u.

**Address for Correspondence:** Ievgeniia Burlaka MD, Bogomolets National Medical University, Department of Pediatrics, Kyiv, Ukraine
**Phone:** +38-097-432-49-44 **E-mail:** evgbur1982@gmail.com **ORCID:** orcid.org/0000-0001-6043-7325

**Conflict of interest:** None declared
**Received:** 21.02.2022
**Accepted:** 10.05.2022

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

J Clin Res Pediatr Endocrinol
2022;14(3):313-323

**Conclusion:** Thus, we hypothesize that T1D pediatric patients with increased PLT count, hyperfiltration and reduced anti-apoptotic defense may represent a group for additional therapeutic interventions, such as antioxidants along with stndard therapies to achieve optimal glycemic control. Within the DN group there was a sub-group with somewhat increased PLT count, high frequency of DKA episodes/year, high MAU, prominent increase in HIF level, prominent disturbances in apoptosis controlling factors BcL-xL and caspase-3 tht may require additional therapeutic interventions, again including antioxidants, but may additionally benefit from anti-apoptotic effectors along with optimal glycemic control, and management of hypertension and albuminuria.
**Keywords:** Early diabetic nephropathy, T1D, hypoxia, HIF-1 alfa, apoptosis, predictors

## Introduction

An estimated 1.1 million people under 20 years of age are affected by type 1 diabetes (T1D) worldwide (1,2). T1D represents 5-10% of the global diabetes burden and is not a disease of childhood alone, with almost half diagnosed in adulthood (3,4). Overall annual increase in T1D is estimated at 3% (2-5%) (5). Diabetic nephropathy (DN) is one of the most common complications of diabetes mellitus, affecting 25 to 40% of patients with T1D. It is the single most common cause of end stage renal disease (ESRD) in adults in the Western world (6,7). DN is marked by pathological changes occurring in the renal glomeruli that lead to the development of albuminuria, hypertension, and progressive decline in renal function (6,7,8).

Traditionally, different factors, such as the duration of diabetes, puberty, age at onset, family history of diabetic complications, family history of insulin resistance, type 1 and 2 diabetes, genetic factors, race/ethnicity, glycemic (metabolic) control, smoking, hyperlipidemia, intrauterine exposure, obesity, pregnancy, and social status have ben identified in terms of risk factors and predictors of T1D complications, including DN (9,10).

An alternative approach, attmpting to identify subgroups of patients with T1D would be to make an evaluation of patients based on different clinical, anamnestic, and pathogenic characteristics. This approach could use a global space-time clustering for cases of T1D. This appraoch has been used to identify possible sex-related differences in response to an infectious agent in patients with T1D (11).

In this study, in addition to the main clinical data, we analyzed pediatric patients with T1D and early DN markers of apoptosis, including proteins belonging to the Bcl-2 family. It is been shown previously that the Bcl-2 family of proteins plays a central role in monitoring the genetic programs of the organism. We also measured hypoxia-inducible factor 1 alfa (HIF-1alfa) in all subjects. The rationale for this was as follows. Hypoxia is present in animal models and may be found as early as three days after the induction of diabetes, predominantly in the medullary region (12). However, comprehensive studies to evaluate the possibility of using clinical markers in combination with markets of apoptosis and hypoxia for T1D and DN stratification in children have not done yet.

The aim of the present study was to evaluate cluster groups of children with T1D and DN, based on levels of transcription factor and marker of intracellular hypoxia including HIF-1alfa and anti-apoptotic factor BcL-xL and the proapoptotic factor, caspase-3, together with basic clinical and laboratory parameters in order to attempt to identify potential subgroups of patients with T1D and DN that may be amenable for additional therapeutic interventions.

## Methods

### Patients

The study included data from 2013 to 2020 with a total of.104 children (62 males and 42 females) with T1DM and early stage of DN followed-up in the Endocrinology unit of Clinical Pediatric Hospital No 6 (Kyiv, Ukraine). The study was approved by the Ethics Committee of the Bogomolets National Medical University (approval No 142). All informed consents were signed by children (≥12 years old) themselves and/or by their parents and kept in medical records. Medical records and data, including anamnesis was analyzed in all patients. All diabetic patients were seen every 3 months and all were on multiple flexible dosing intervals of insulin treatment. Chronological age, diabetes duration, weight, height, body mass index (BMI), blood pressure, hemoglobin A1c (Hb1Ac), serum cholesterol, complete blood count, urinalysis, and urine albumin excretion was measured and recorded at each visit to hospital.

Patients with T1D and without signs of DN with urinary albumin excretion within physiological range prior the study inclusion and at each follow-up visit were designated group T1D (n = 57). Disease duration in this group was ≥1 year.

The DN group (n = 48) were children with DN observed during 1 year after the first documented episode of albuminuria. Diagnosis of the DN was based on the measurement of abnormal levels of urinary albumin in diabetic patients after the exclusion of other causes of albuminuria. Two out of three samples falling within the microalbuminuria (MAU) (30 to 300 mg of albumin/24 h)

or macroalbuminuria (more than 300 mg of albumin/24 h) range confirm the presence of DN. Urinary MAU/albumin excretion measured in 24-hour urine collection samples using basic conventional technique established in Clinical Pediatric Hospital No 6. Causes of albuminuria were excluded in all patients in the DN group.

Excludion criteria included severe chronic and acute diseases, such as chronic inflammatory diseases, autoimmune diseases, transplantation, viral hepatitis B or C, liver cirrhosis, or other severe liver diseases, acute and chronic gastrointestinal diseases, previous acute kidney injury, chronic kidney disease (CKD), major surgery within 12 months before study, AIDS, heart disease, and cancer.

Glomerular filtration rate (GFR) was used to assess kidney function. The Schwartz formula for children and adolescents 1 to 17 years old was used (13). The demographic and clinical characteristics of the patients included in the study is shown in Table 1.

### Immunoblotting for Detection of HIF-1alfa, BcL-xL, Caspase-3

Plasma samples were used to measure markers of apoptosis and intracellular hypoxia response. Proteins suspended in Laemmli sample buffer were resolved in polyacrylamide gels by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Membranes were then blocked in 5% non-fat milk in TBS-T (136 mM NaCl, 10 mM Tris, 0.05% Tween 20) and immunoblotted using antibodies specific for Bcl-xL and HIF-1α, and caspase-3 (Cell Signaling Technology, Danvers, MA USA) for 1 hour at room temperature. An actin mouse monclonal antibody was used as a loading control. After three washes with TBS-T, the membranes were incubated with secondary anti-rabbit or anti-mouse antibodies labeled with horseradish peroxidase for 1 hour at room temperature. Membranes were washed three times with TBS-T. Protein bands were visualized by Enhanced chemiluminescence substrate. Quantification of the protein content was done by densitometric analysis.

**Table 1. Clinical characteristics of patients**

| Parameter, mean ± SEM | T1D (n = 57) | DN group (T1D with diabetic nephropathy) (n = 47) |
|---|---|---|
| Age, years | 12.74 ± 0.77 | 13.25 ± 0.56 |
| Boys/girls | 29/28 | 33/14 |
| Boys, age, years | 11.73 ± 0.82 | 12.82 ± 0.76 |
| Girls, age, years | 13.85 ± 1.33 | 14.2 ± 0.66 |
| Duration of T1D | 4.9 ± 0.5 | 6.0 ± 051 |
| BMI, kg/m$^2$ | 18.75 ± 0.63 | 19.72 ± 0.55 |
| Boys, BMI, kg/m$^2$ | 18.14 ± 0.63 | 19.65 ± 0.72 |
| Girls, BMI, kg/m$^2$ | 19.42 ± 1.13 | 20.05 ± 0.9 |
| Systolic blood pressure, mmHg | 106.5 ± 1.44 | 126.4 ± 1.34*** |
| Diastolic blood pressure, mmHg | 71.02 ± 0.88 | 71.94 ± 1.11 |
| PLT, 10$^7$/L | 267.6 ± 8.14 | 262,9 ± 8,83 |
| ESR, mm/h | 4.8 ± 0.13 | 10.45 ± 0.53** |
| Albumin/globulin ratio | 1.26 ± 0.04 | 1.00 ± 0.03** |
| Serum cholesterol, mMol/L | 4.58 ± 0.15 | 5.83 ± 0.14** |
| GFR, mL/min/1.73 m$^2$ | 135.5 ± 24.21 | 85.87 ± 2.19*** |
| Hb1Ac, % | 9.41 ± 0.3 | 10.22 ± 9.55 |

**p < 0.01, ***p < 0.001.
SEM: standard error of the mean, T1D: type 1 diabetes, DN: diabetic nephropathy, BMI: body mass index, PLT: platelets, ESR: erythrocyte sedimentation rate, GFR: glomerular filtration rate, Hb1Ac: hemoglobin A1c

### Statistical Analysis

The data are expressed as means ± standard error of the mean. ANOVA followed by post-hoc Kruskal-Wallis test for multiple comparisons was used to test significance of differences. Data was analyzed using GraphPad Prism 9.0 Software for Windows (San Diego, CA, USA). Two-step clustering was done using Statistica 10.0 software. An intelligent clustering method in which the optimal clustering number is automatically determined was performed. This identifies clusters by two processes: first, preclustering, followed by hierarchical clustering. Hierarchical algorithms were used to estimate the optimal clustering number based on the silhouette width, the calculation of the distance using the log-likelihood and clustering in accordance with Schwarz's Bayesian criterion. P values $<0.05$ were considered statistically significant.

## Results

### Clinical Characteristics of Patients

### Identification and Characteristics of Three Clusters by Remodeling the Cluster Analysis Based on Fourteen Variables in Children with T1D

The clustered results, based on nine variables - disease course, age, BMI, systolic blood pressure (SBP), platelet (PLT) count, erythrocyte sedimentation rate (ESR), albumin/globulin ratio, serum cholesterol, Hb1Ac, number of diabetic ketoacidosis (DKA) episodes, GFR, HIF-1alfa, Bcl-xL, caspase-3 are shown as three subgroups in T1D patients (Figure 1).

These cluster groups were designated cluster I, cluster II, and cluster III. Disease duration and mean age values did not show any difference between the clusters (Figure 2A, 2B). The mean BMI was also similar in clusters I-III (Figure 2C). Mean SBP values did not show statistical differences between the clusters (Figure 2D).

PLT count, ESR, albumin/globulin ratio, serum cholesterol level were selected as basic laboratory markers. The mean PLT count in cluster I was $344.9 \pm 7.88 \cdot 10^9$/L, in cluster II - $257.4 \pm 3.02 \cdot 10^9$/L ($p < 0.01$) and cluster III - $205.1 \pm 12.52 \cdot 10^9$/L ($p < 0.001$ - cluster I vs. cluster II value and $p < 0.0001$ - cluster I vs. cluster III) (Figure 3A). Similar mean ESR values were found in the three clusters ($7.54 \pm 1.53$ mm/h, $6.06 \pm 0.83$ mm/h and $8.22 \pm 2.5$ mm/h, respectively; $p > 0.05$) (Figure 3B). The albumin/globulin ratio did not show difference between cluster I, II and III ($1.2 \pm 0.05$, $1.26 \pm 0.05$ and $1.32 \pm 0.16$, respectively; $p > 0.05$) (Figure 3C). Furthermore, the mean serum cholesterol level was also similar between the clusters ($4.85 \pm 0.18$ mMol/L in cluster I, $4.49 \pm 0.17$ mMol/L in cluster II and $4.77 \pm 0.62$ mMol/L in cluster III; $p > 0.05$) (Figure 3D).

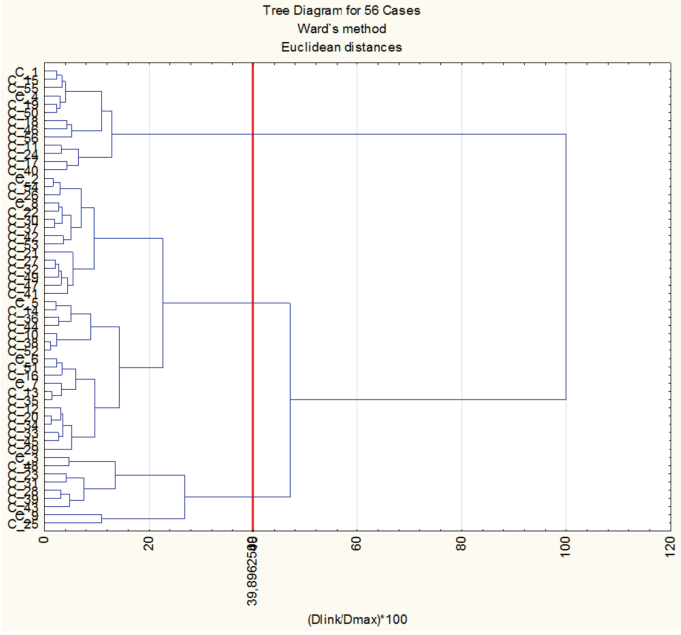


**Figure 1.** Identification of three clusters by remodeling the cluster analysis based on fourteen variables in children with type 1 diabetes

Hb1Ac, number of DKA episodes, and GFR were selected as markers of T1D compensation and kidney function. The mean Hb1Ac value did not differ between the clusters (Figure 4A). However, the average number of DKA episodes/year in cluster II was somewhat higher than in cluster I ($2.12 \pm 0.26$ episodes/year vs. $1.91 \pm 0.42$ episodes/year) but this was not significant. In cluster III the mean number of DKA episodes per year was $2.19 \pm 0.31$ ($p > 0.05$ - cluster I vs. cluster II and III) (Figure 4B). Finally, GFR as a direct indicator of kidney function was investigated in all subjects. The mean GFR value was similar in cluster II and III ($98.13 \pm 2.99$ and $91.9 \pm 5.82$ mL/min/1.73 m$^2$, respectively; $p > 0.05$). Cluster I GFR value was $124.5 \pm 8.86$ mL/min/1.73 m$^2$ which was significantly higher compared to cluster II and cluster III ($p < 0.05$) (Figure 4C).

The expression of proapoptotic factor, caspase-3, anti-apoptotic factor BcL-xL, and the marker of intracellular hypoxia, HIF-1alfa were also analyzed. HIF-1alfa was selected as a marker of chronic hypoxia but was similar in cluster I, II and III ($165.4 \pm 3.83$ a.u., $165.0 \pm 1.6$ a.u. and $158.2 \pm 3.19$ a.u., respectively; $p > 0.05$) (Figure 5A). The Bcl-xL level in cluster I was $144.9 \pm 2.35$ a.u. which is significantly lower compared to the value in cluster III at $160.0 \pm 2.4$ a.u. ($p < 0.001$). In addition this value in cluster II was $140.6 \pm 1.57$ and was found to be significantly lower

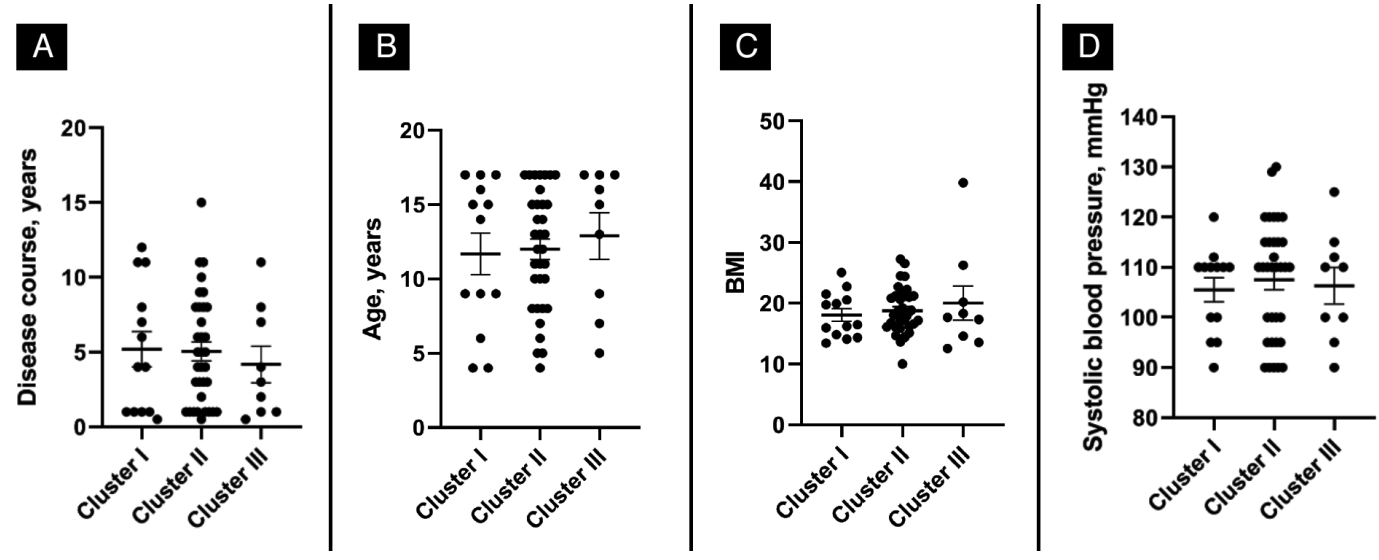


**Figure 2.** Disease course (A), age (B), BMI (C), SBP (D) in cluster groups of children with T1D. Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*BMI: body mass index, SBP: systolic blood pressure, SEM: standard error of the mean, T1D: type 1 diabetes*

compared to cluster III ($p < 0.05$) (Figure 5B). The level of caspase-3 in cluster I, cluster II and cluster III was similar at $137.7 \pm 3.28$ a.u., $137.6 \pm 2.13$ a.u. and $136.0 \pm 2.99$ a.u., respectively ($p > 0.05$) (Figure 5C).

### Identification and Characteristics of the Three Clusters by Remodeling the Cluster Analysis in Children with Early DN

The clustered groups were designated based on fourteen variables, including age, BMI, SBP, PLT, ESR, albumin/globulin ratio, serum cholesterol, Hb1Ac, number of DKA episodes, GFR, MAU, HIF-1alfa, Bcl-xL, caspase-3 in children with early DN (Figure 6).

No difference was documented in mean age (Figure 7A), mean BMI, and SBP values between clusters in the DN group (Figure 7B, 7C) when compared clusters I-III.

The mean PLT in cluster I was $311. \pm 12.05 \cdot 10^9$/L, which is higher when compared to cluster II ($260.4 \pm 11.12 \cdot 10^9$/L; $p < 0.01$) and cluster III ($273.4 \pm 8.05 \cdot 10^9$/L; $p < 0.05$) (Figure 8A). The ESR level was similar in the three clusters at $10.18 \pm 1.55$ mm/h, $9.36 \pm 1.22$ mm/h and $8.91 \pm 0.92$ mm/h, respectively ($p > 0.05$) (Figure 8B). The albumin/globulin ratio was also not different between the three clusters at $1.55 \pm 0.04$, $1.12 \pm 0.05$ and $1.19 \pm 0.05$, for clusters I, II and III respectively ($p > 0.05$) (Figure 8C).

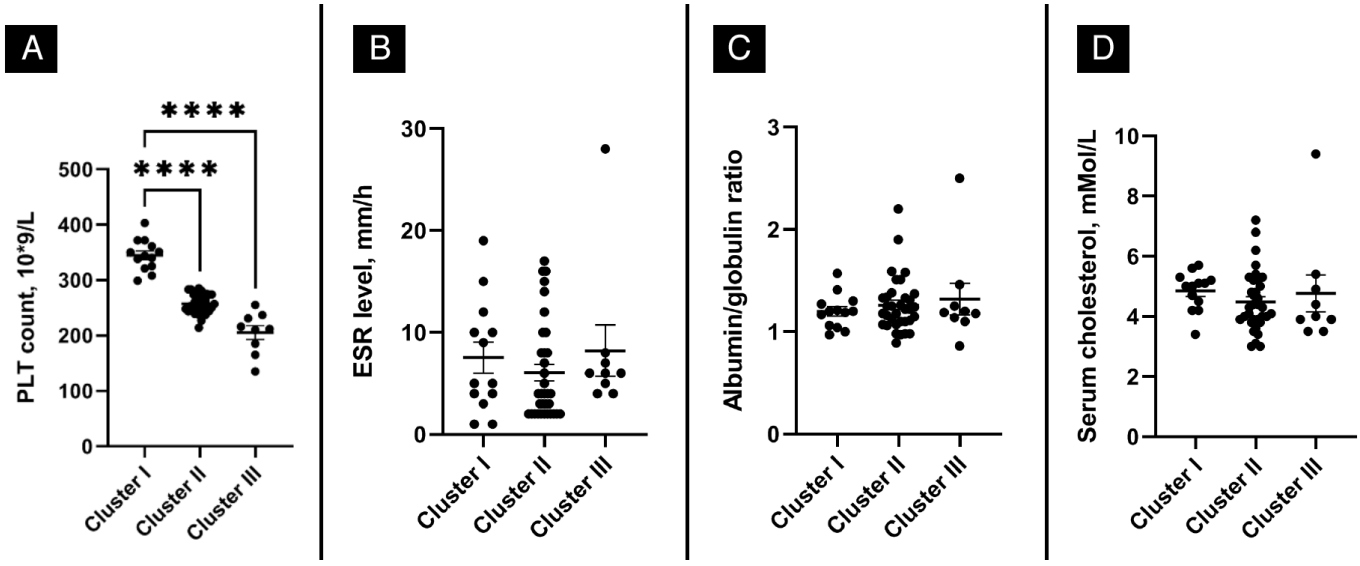


**Figure 3.** PLT count (A), ESR (B), albumin/globulin ratio (C) and serum cholesterol (D) levels in cluster groups of children with T1D (****$p < 0.0001$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*SEM: standard error of the mean, T1D: type 1 diabetes, PLT: platelets, ESR: erythrocyte sedimentation rate*

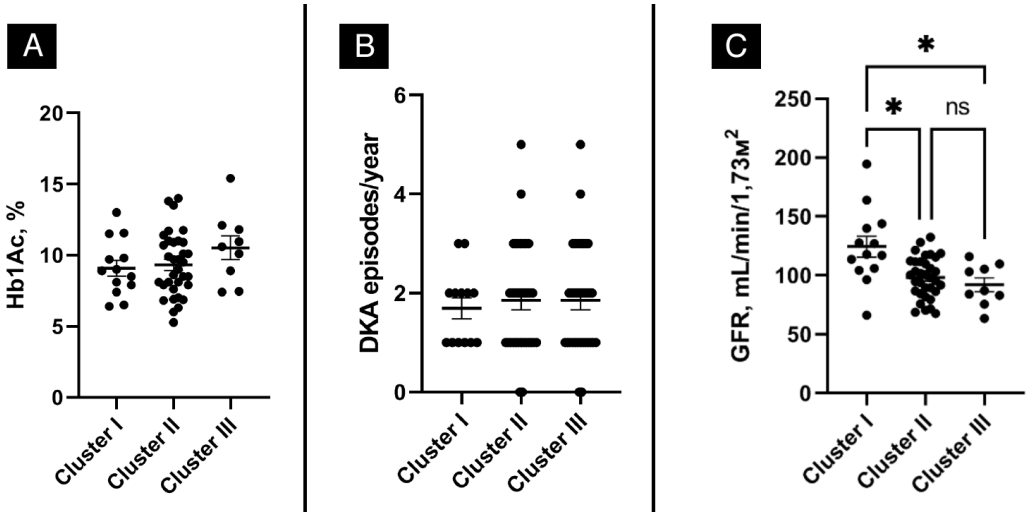


**Figure 4.** Hb1Ac (A), number of DKA episodes (B), GFR (C) levels in cluster groups of children with T1D. Ns: not significantly different (*$p < 0.05$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*Hb1Ac: hemoglobin A1c, DKA: diabetic ketoacidosis, GFR: glomerular filtration rate, SEM: standard error of the mean, T1D: type 1 diabetes*

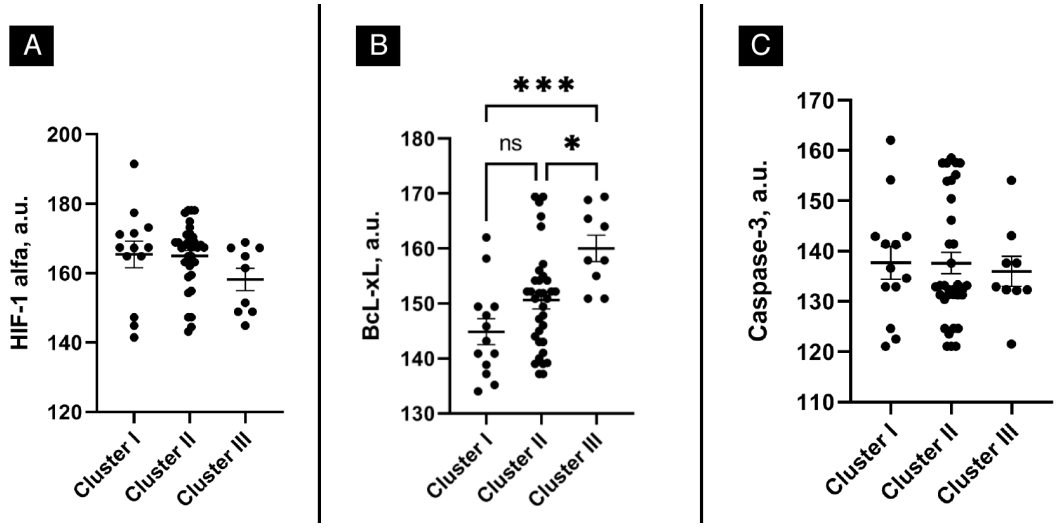


**Figure 5.** HIF1-alfa (A), BcL-xL (B), caspase-3 (C) levels in cluster groups of children with T1D. Ns: not significantly different (*$p < 0.05$, *$p < 0.001$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*SEM: standard error of the mean, T1D: type 1 diabetes*

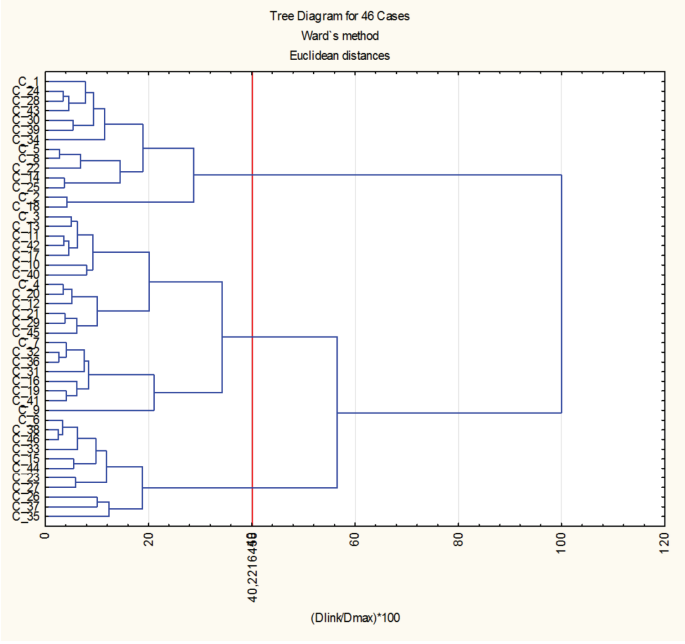


**Figure 6.** Identification of three clusters by remodeling the cluster analysis based on fourteen variables in children with early DN

*DN: diabetic nephropathy*

Furthermore, the serum cholesterol level was also similar at 5.86 ± 0.23 mMol/L in cluster I, 5.66 ± 0.31 mMol/L in cluster II and 5.84 ± 0.19 mMol/L in cluster III ($p > 0.05$) (Figure 8D).

The mean Hb1Ac was not different between the three clustering groups; 10.81 ± 0.73 % in cluster I, 9.92 ± 0.7 % in cluster II, and 9.83 ± 0.42 % in cluster III ($p > 0.05$) (Figure 9A). The average number of DKA episodes/year in cluster I was 4.81 ± 0.26 episodes/year, in cluster II it was 3.21 ± 0.42 episodes/year and in cluster III it was 4.29 ± 0.31 episodes/year ($p > 0.05$) (Figure 9B).

GFR as a direct indicator of kidney function was evaluated in all children with T1D. The mean GFR value did not show any difference between cluster I, II and III at 87.57 ± 3.8, 85.05 ± 3.58 and 83.62 ± 3.71 mL/min/1.73 $m^2$, respectively ($p > 0.05$) (Figure 9C). MAU excretion as a direct indicator of kidney damage was analyzed in all children with DN. The mean MAU value in cluster I was 112.0 ± 10.12 mg/24 h and was significantly higher compared to cluster II 38.25 ± 6.32 mm/h ($p < 0.001$) and cluster III at 35.64 ± 2.82 mm/h ($p < 0.001$) (Figure 9D).

The expression of HIF-1alfa, BcL-xL and caspase-3 was analyzed in clusters of children with DN. The values of HIF-1 alfa were similar in cluster II and cluster III at 182.5 ± 5.11 a.u. and 185.2 ± 3.28 a.u., respectively ($p > 0.05$). However, HIF-1 alfa in cluster I was significantly higher than in clusters II and II at 200.5 ± 3.49 a.u. ($p < 0.05$) (Figure 10A). The mean value of Bcl-xL in cluster I was 128.8 ± 3.1 a.u. which was significantly lower compared to the cluster II value of 146.3 ± 3.27 a.u. ($p < 0.05$) but did not differ from the cluster III value of 137.2 ± 2.67 a.u. ($p > 0.05$) (Figure 10B). Caspase-3 results were similar between cluster I, cluster II and cluster III at 159.6 ± 5.5 a.u., 137.7 ± 3.64 a.u. and 146.3 ± 2.67 a.u., respectively ($p > 0.05$) (Figure 10C).

## Discussion

Recent trends have indicated that the incidence of diabetes is increasing rapidly worldwide, with a dramatic increase in

prevalence in the Middle Eastern countries, among both adults and children (14,15). DN is the leading cause of end-stage renal disease worldwide. Chronic hyperglycemia and high blood pressure are the main risk factors for the development of DN. In general, screening for MAU should be performed annually, starting five years after diagnosis in T1D.

The pathogenesis of DN development and progression is complex and multifactorial with the involvement of many pathways and mediators (16). Conventionally, the developmental mechanism of DN is the result of abnormal homeostasis, which includes hemodynamic abnormalities, metabolic disorders, and hormone synthesis, such as Ang-II. The renin-angiotensin-aldosterone system, advanced glycation end product (AGE) formation, activation of transforming growth factor-β1, connective tissue growth factor, protein kinase C, mitogen-activated protein kinase, and reactive oxygen species are important pathways to the development and progression of DN (17). This is why the exact pathogenic mechanism and molecular incidence of DN are still not fully understood and the contribution of each pathway in inducing DN is not clear and thus the early identification of risk groups is challenging.

As with many other CKD, the diagnosis of DN is based on changes in urinary albumin excretion rate and GFR.

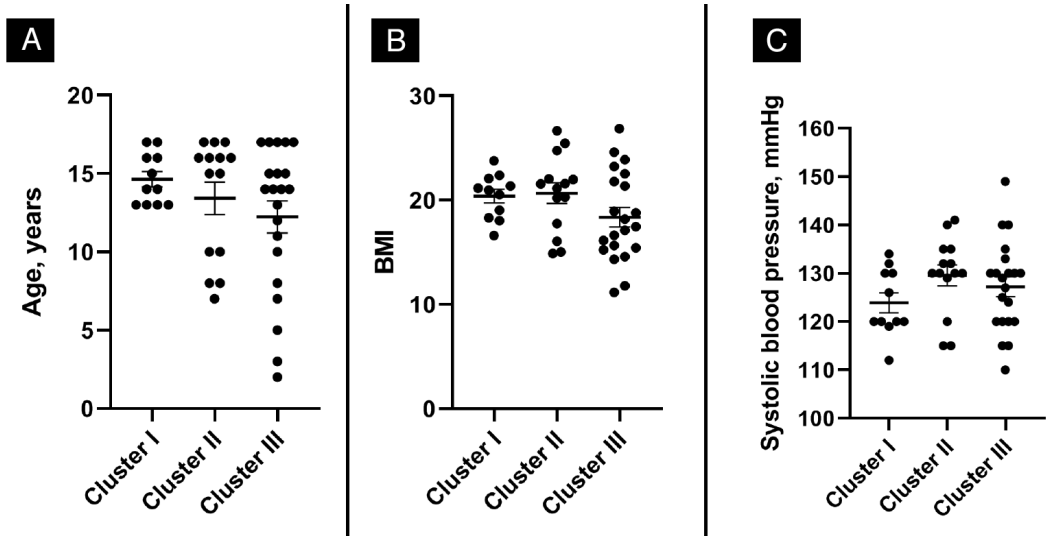


**Figure 7.** Age (A), BMI (B), SBP (C) in cluster groups of children with early DN. Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*BMI: body mass index, SBP: systolic blood pressure, DN: diabetic nephropathy, SEM: standard error of the mean*

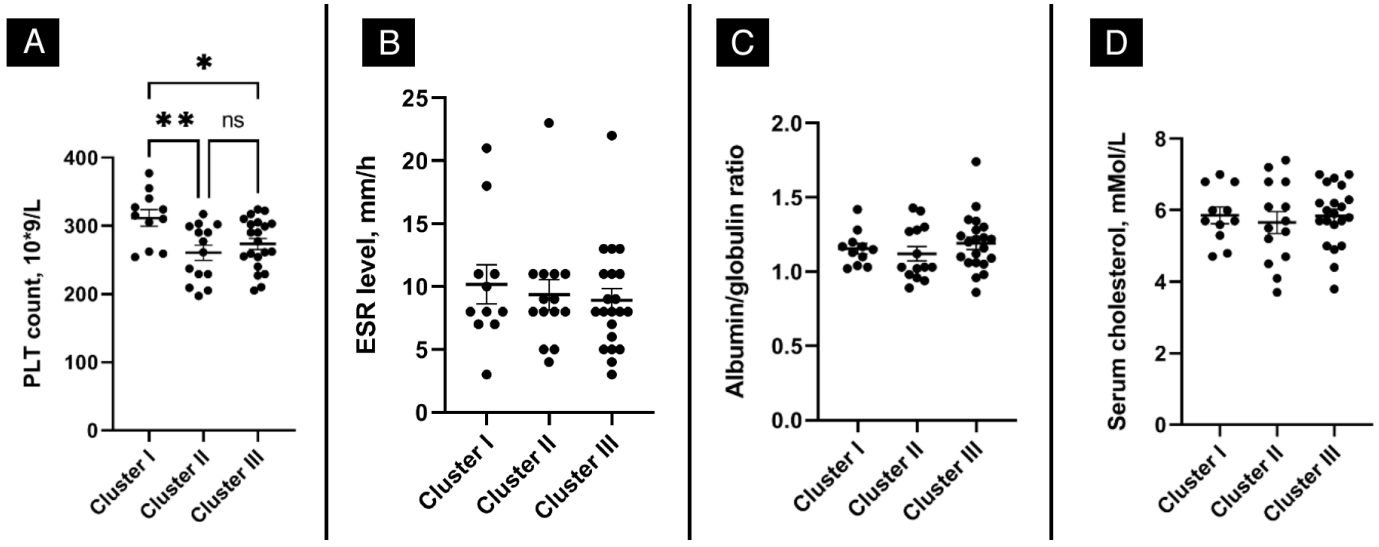


**Figure 8.** PLT count (A), ESR (B), albumin/globulin ratio (C) and serum cholesterol (D) levels in cluster groups of children with early DN (*$p < 0.05$, *$p < 0.01$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*PLT: platelets, ESR: erythrocyte sedimentation rate, DN: diabetic nephropathy, SEM: standard error of the mean*

Structural changes may be observed in kidney biopsies as early as the first few years after the onset of diabetes, but the disease has a long "silent period" in its development (18). Thus, our current understanding of the trajectory of DN in children and adolescents suggests that advanced CKD and kidney failure take decades to develop after the onset/diagnosis of diabetes, which means that the data on the prevalence and time course of these outcomes in childhood-onset diabetes is largely derived from adult studies (19,20). This presents a dilemma for any rigorous study of diabetic kidney disease (DKD) in children and adolescents because understanding any aspect of DN, for example biomarkers, risk factors for progression, and assessment of response to interventions, has had to rely on intermediate outcomes, such as albuminuria, and hyperfiltration. This requires identification of risk hroups of patients based on conventional clinical tests results and novel pathogenic biomarkers.

The lack of reliable surrogate markers for DN progression during childhood and adolescence makes identification of

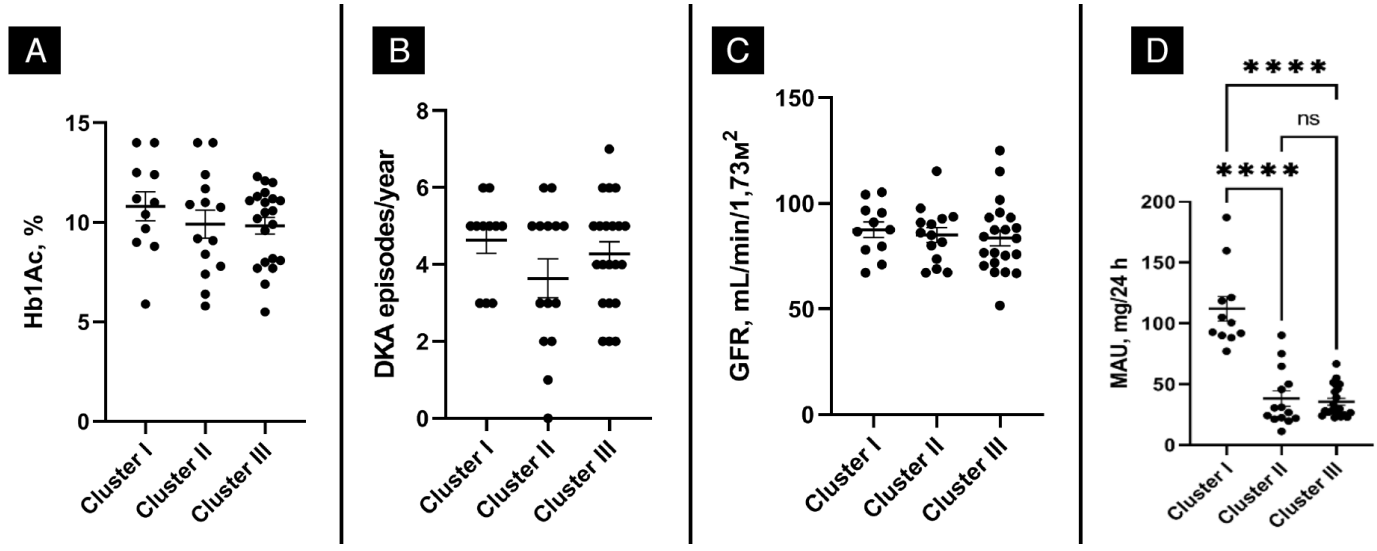


**Figure 9.** Hb1Ac (A), number of DKA episodes (B), GFR (C), MAU (D) levels in cluster groups of children with early DN. Ns: not significantly different, (****$p < 0.0001$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*Hb1Ac: hemoglobin A1c, DKA: diabetic ketoacidosis, GFR: glomerular filtration rate, MAU: microalbuminurea, SEM: standard error of the mean, DN: diabetic nephropathy*

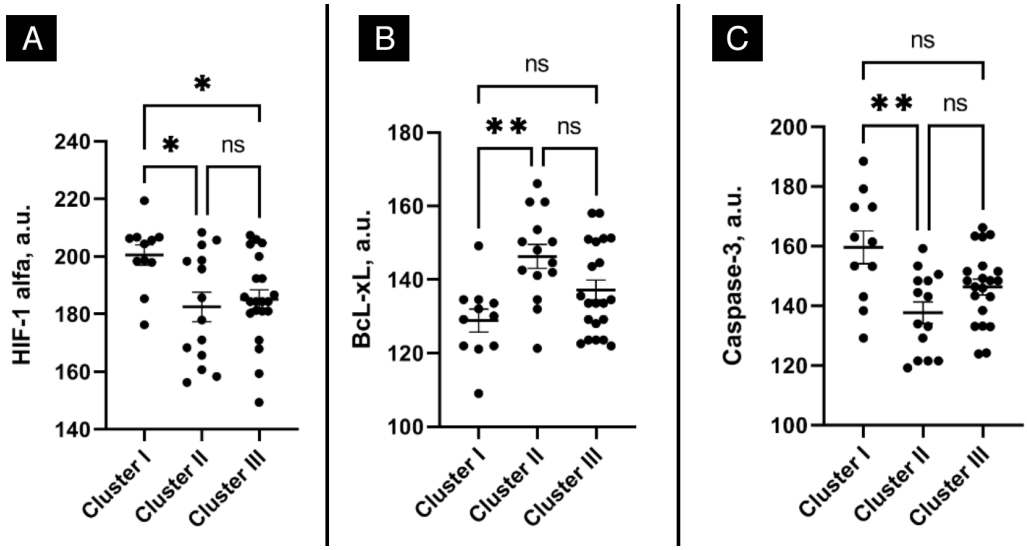


**Figure 10**. HIF1-alfa (A), BcL-xL (B), caspase-3 (C) levels in cluster groups of children with early DN. Ns: not significantly different, (*$p < 0.05$, *$p < 0.01$). Histograms represent means ± SEM. Statistical analysis performed using the post-hoc Kruskal-Wallis test

*DN: diabetic nephropathy*

novel markers of early disease in youth even more critical than it is in adults. Most published studies report cross-sectional associations between various urinary/serum protein biomarkers and intermediate outcomes, such as albuminuria, with a smaller number of studies examining these associations using longitudinal data.

Rare studies are notable in bypassing the reliance on these flawed surrogate markers and examining the association between putative biomarkers, such as plasma AGEs or plasma bradykinin with early kidney structural changes in youth with T1D. In adults, serum tumor necrosis factor receptor 1 (TNFR1) and TNFR2 have been found to be associated with the early structural changes of DN as well as with DN progression, highlighting the contribution of inflammatory pathways to the disease process (21). Other potential biomarkers for adult DN are urinary neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, N-acetyl-β-D-glucosaminidase, and liver fatty acid-binding protein (LFABP) (22,23). None of these putative markers or others is currently a part of routine clinical care in adult or pediatric DKD.

Therefore, the focus of the present study was stratification of children with T1D and early DN using conventional laboratory markers in combination with markers of apoptosis and chronic hypoxia.

We chose members of the Bcl-2 family BcL-xL and caspase-3 as markers of apoptosis. The Bcl-2 family has long been identified for its role in apoptosis regulation. In an *in vivo* model, Wada et al. (24) showed that puromycine-induced podocyte apoptosis was p53 dependent and associated with changes in Bcl-2-related proteins and apoptosis inducing factor AIF translocation. The protective effects of dexamethasone on PA-induced apoptosis were associated with decreasing p53, increasing Bcl-xL, and inhibition of AIF translocation (25).

We measured the level of HIF-1alfa in all patients with T1D and early DN which is an important transcriptional factor regulating many cellular functions. HIF-1 is a heterodimer composed of the rate limiting factor HIF1α and the constitutively expressed HIF-1β (26). DN is associated with chronic, low-grade inflammation under the persistent influence of MAU and glucose (25). Hypoxia can induce apoptosis by causing hyperpermeability of the inner mitochondrial membrane, which leads to the release of cytochrome C and apoptosis induction (27).

Our results show that all examined children with T1D may be divided into three groups based on fourteen variables, - disease course, age, BMI, SBP, PLT count, ESR, albumin/globulin ratio, serum cholesterol, Hb1Ac, number of DKA episodes, GFR, HIF-1alfa, Bcl-xL, caspase-3 levels. Cluster I can be defined as a risk group characterized by somewhat higher PLT, increased GFR up to hyperfiltration level and decreased anti-apoptotic defense. Cluster II and cluster III did not show these characteristics.

Children with DN may be divided into three groups based on age, BMI, SBP, PLT count, ESR, albumin/globulin ratio, serum cholesterol, Hb1Ac, number of DKA episodes, GFR, MAU, HIF-1alfa, Bcl-xL, caspase-3 levels. Cluster I was found as the most different and its characteristics are PLT count, frequency of DKA episodes, mean MAU in 254 hour urine collection, and levels of HIF, BcL-xL and caspase-3.

We speculate that cluster I in the T1D group, characterized by somewhat of an increase in PLT, hyperfiltration and reduced anti-apoptotic defense should be considered as a potential risk group for further complications, including DN and cardiovascular events. Previously, we have shown that

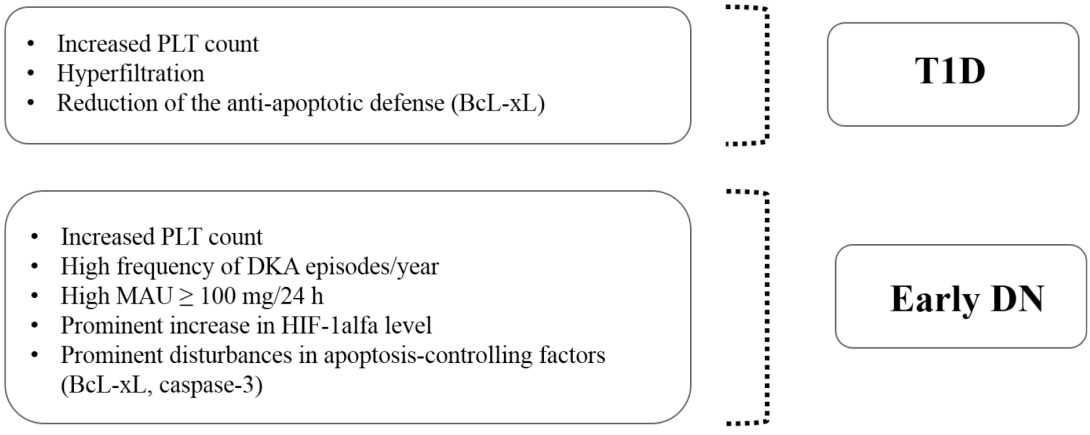


**Figure 11**. Summarized scheme of the parameters of the clustered risk groups of children with T1D and early DN

*DN: diabetic nephropathy, T1D: type 1 diabetes, PLT: platelets, DKA: diabetic ketoacidosis*

children with T1D have increased GFR (28). This finding is in line with other research showing that a 25-50 % elevation in the GFR is seen early in the course in up to one-half of patients with T1D, an abnormality that is exaggerated after ingestion of a protein load. Glomerular hypertrophy and increased kidney size typically accompany the rise in GFR (29,30). PLT may be a factor possibly contributing to future cardiovascular events as well. It has been shown that enhanced PLT reactivity is considered a main determinant of the increased atherothrombotic risk of diabetic patients. Thrombopoietin, a humoral growth factor able to stimulate megakaryocyte proliferation and differentiation, also modulates the response of mature PLT by enhancing both activation and binding to leukocytes in response to different agonists (31). Cluster I in the DN group was found to have a somewhat increased PLT count, high frequency of DKA episodes/year, high MAU, prominent increase in HIF level, and prominent disturbances in apoptosis controlling factors BcL-xL and caspase-3. We speculate that in addition to the pathogenic effects from modestly incresed PLT and DKA-related cardiovascular and circulatory disorders due to poor metabolic control and glycemic variability, albuminuria causes additional stimulating effect on apoptosis. Albuminuria is a potent apoptotic agent. Albumin uptake in primary rat renal epithelial cells is accompanied by a time- and dose-dependent mitochondrial accumulation of the apoptotic factor Bax, down-regulation of the antiapoptotic factor Bcl-xL and mitochondrial membrane depolarization (32). A summarized scheme of the parameters of the risk groups of children with T1D and early DN is given in Figure 11.

### Study Limitations

This study has certain limitations that must be acknowledged. Our pilot study was cross-sectional, at a single center with modest patient numbers. The strength is that enrolled patients were studied for the full range of clinical, laboratory, and anamnestic markers in parallel with markers of hypoxia and apoptosis measurement.

## Conclusion

Thus, we hypothesize that T1D pediatric patients with increased PLT, hyperfiltration and reduced anti-apoptotic defense may constitute a group requiring therapeutic interventions, such as antioxidants along with conventional treatment and optimal glycemic control. Within the DN group, there was a sub-group with somewhat increased PLT count, high frequency of DKA episodes/year, high MAU, prominent increase in HIF-1alfa level, prominent disbalance in level of apoptosis controlling factors BcL-xL and caspase-3 which may also require additional therapeutic interventions, once again including antioxidants, but may also warrant anti-apoptotic effectors along with optimal glycemic control, management of hypertension and albuminuria.

### Acknowledgement

We acknowledge the assistance of Endocrinology unit of the Clinical Pediatric Hospital No 6 (Kyiv, Ukraine).

### Ethics

**Ethics Committee Approval:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Bogomolets Medical University (protocol code: 142, date: 22.02.2022).

**Informed Consent:** Informed consent was obtained from all subjects involved in the study.

**Peer-review:** Externally peer-reviewed.

**Financial Disclosure:** The author declared that this study received no financial support.

## References

1. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, Ogle GD. Worldwide Estimates of Incidence, Prevalence and Mortality of Type 1 Diabetes in Children and Adolescents: Results From the International Diabetes Federation Diabetes Atlas, 9th Edition. Diabetes Res Clin Pract 2019;157:107842. Epub 2019 Sep 10
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and Regional Diabetes Prevalence Estimates for 2019 and Projections for 2030 and 2045: Results From the International Diabetes Federation Diabetes Atlas, 9(Th) edition. Diabetes Res Clin Pract 2019;157:107843. Epub 2019 Sep 10
3. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 Diabetes. Lancet 2014;383:69-82. Epub 2013 Jul 26
4. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and Phenotype of Type 1 Diabetes in the First Six Decades of Life: A Cross-Sectional, Genetically Stratified Survival Analysis From UK Biobank. Lancet Diabetes Endocrinol 2018;6:122-129. Epub 2017 Nov 30
5. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of Type 1 Diabetes. Endocrinol Metab Clin North Am 2010;39:481-497.
6. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and risk factors. J Nephropharmacol 2015;5:49-56.
7. Piccoli GB, Grassi G, Cabiddu G, Nazha M, Roggero S, Capizzi I, De Pascale A, Priola AM, Di Vico C, Maxia S, Loi V, Asunis AM, Pani A, Veltri A. Diabetic Kidney Disease: A Syndrome Rather Than a Single Disease. Rev Diabet Stud 2015;12:87-109.
8. Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis 2014;7:361-381.
9. Traversi D, Rabbone I, Scaioli G, Vallini C, Carletto G, Racca I, Ala U, Durazzo M, Collo A, Ferro A, Carrera D, Savastio S, Cadario F, Siliquini R, Cerutti F. Risk factors for type 1 diabetes, including environmental,

behavioural and gut microbial factors: a case-control study. Sci Rep 2020;10:17566.

10. Wu YL, Ding YP, Gao J, Tanaka Y, Zhang W. Risk Factors and Primary Prevention Trials for Type 1 Diabetes. Int J Biol Sci 2013;9:666-679.
11. McNally RJ, Pollock R, Court S, Begon M, Cheetham TD. Space-time clustering analyses of type 1 diabetes in children from north-east England: Support for an infectious aetiology?. Environ Health 2009;8(Suppl 1):14.
12. Catrina SB, Zheng X. Hypoxia and hypoxia-inducible factors in diabetes and its complications. Diabetologia 2021;64:709-716. Epub 2021 Jan 26
13. Fadrowski JJ, Furth SL. GFR Estimation in Children: Questions and Answers (and Questions). Clin J Am Soc Nephrol 2011;6:1810-1812. Epub 2011 Jul 22
14. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, Song X, Ren Y, Shan PF. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Sci Rep 2020;10:14790.
15. Liu J, Ren ZH, Qiang H, Wu J, Shen M, Zhang L, Lyu J. Trends in the incidence of diabetes mellitus: results from the Global Burden of Disease Study 2017 and implications for diabetes mellitus prevention. BMC Public Health 2020;20:1415.
16. Tavafi M. Complexity of diabetic nephropathy pathogenesis and design of investigations. J Renal Inj Prev 2013;2:59-62.
17. Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int 2021;2021:1497449.
18. Di Vincenzo A, Bettini S, Russo L, Mazzocut S, Mauer M, Fioretto P. Renal structure in type 2 diabetes: facts and misconceptions. J Nephrol 2020;33:901-907. Epub 2020 Jul 12
19. Yang CC, Lin CH, Wang NK, Lai CC, Lo FS; Chang Gung Juvenile Diabetes Eye Study Group. Risk Factors Associated With the Development of Nephropathy 10 Years After Diagnosis in Taiwanese Children With Juvenile-Onset Type 1 Diabetes-A Cohort Study From the CGJDES. Front Endocrinol (Lausanne) 2018;9:429.
20. Lopez LN, Wang W, Loomba L, Afkarian M, Butani L. Diabetic kidney disease in children and adolescents: an update. Pediatr Nephrol 2021.
21. Gohda T, Niewczas MA, Ficociello LH, Walker WH, Skupien J, Rosetti F, Cullere X, Johnson AC, Crabtree G, Smiles AM, Mayadas TN, Warram JH, Krolewski AS. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol 2021;23:516-524. Epub 2012 Jan 19
22. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL, Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS, Bonventre JV. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol 2014;25:2177-2186. Epub 2014 Jun 5
23. Hsu CY, Xie D, Waikar SS, Bonventre JV, Zhang X, Sabbisetti V, Mifflin TE, Coresh J, Diamantidis CJ, He J, Lora CM, Miller ER, Nelson RG, Ojo AO, Rahman M, Schelling JR, Wilson FP, Kimmel PL, Feldman HI, Vasan RS, Liu KD; CRIC Study Investigators; CKD Biomarkers Consortium. Urine biomarkers of tubular injury do not improve on the clinical model predicting chronic kidney disease progression. Kidney Int 2017;91:196-203. Epub 2016 Oct 28
24. Wada T, Pippin JW, Marshall CB, Griffin SV, Shankland SJ. Dexamethasone Prevents Podocyte Apoptosis Induced by Puromycin Aminonucleoside: Role of p53 and Bcl-2-Related Family Proteins. J Am Soc Nephrol 2005;16:2615-2625. Epub 2005 Jun 29
25. Zheng W, Guo J, Liu ZS. Effects of metabolic memory on inflammation and fibrosis associated with diabetic kidney disease: an epigenetic perspective. Clin Epigenetics 2021;13:87.
26. Gunton JE. Hypoxia-inducible factors and diabetes. J Clin Invest 2020;130:5063-5073.
27. Greijer AE, van der Wall E. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 2004;57:1009-1014.
28. Burlaka I, Maidannyk V. Early functional and metabolic disorders in children with type I diabetes mellitus and diabetic nephropathy. Pediatr Endocrinol Diabetes Metab 2021;27:170-177.
29. Bank N. Mechanisms of diabetic hyperfiltration. Kidney Int 1991;40:792-807.
30. Tuttle KR, Bruton JL, Perusek MC, Lancaster JL, Kopp DT, DeFronzo RA. Effect of strict glycemic control on renal hemodynamic response to amino acids and renal enlargement in insulin-dependent diabetes mellitus. N Engl J Med 1991;324:1626-1632.
31. Bosco O, Vizio B, Gruden G, Schiavello M, Lorenzati B, Cavallo-Perin P, Russo I, Montrucchio G, Lupia E. Thrombopoietin Contributes to Enhanced Platelet Activation in Patients with Type 1 Diabetes Mellitus. Int J Mol Sci 2021;22:7032.
32. Burlaka I, Nilsson LM, Scott L, Holtbäck U, Eklöf AC, Fogo AB, Brismar H, Aperia A. Prevention of apoptosis averts glomerular tubular disconnection and podocyte loss in proteinuric kidney disease. Kidney Int 2016;90:135-148. Epub 2016 May 21

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2022.2022-2-10

J Clin Res Pediatr Endocrinol 2022;14(3):324-333

# Psychometric Properties of the Turkish Version of the Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes

**Aslı Demirtaş[1], Burcu Aykanat Girgin[2], Ayla Güven[1], Heves Kırmızıbekmez[3]**

*[1]Zeynep Kamil Maternity and Children Diseases Training and Research Hospital, Clinic of Pediatric Endocrinology, İstanbul, Turkey*
*[2]University of Health Sciences Turkey, Hamidiye Faculty of Nursing, Department of Pediatric Nursing, İstanbul, Turkey*
*[3]İstanbul Ümraniye Training and Research Hospital, Clinic of Pediatric Endocrinology, İstanbul, Turkey*

### What is already known on this topic?

In adolescents with type 1 diabetes mellitus (T1DM), diabetes resilience is reported to reduce the negative emotions associated with diabetes, increase self-efficacy behavior in diabetes management, and facilitate metabolic control. At present, there is no validated assessment tool to measure diabetes-specific resilience and strengths of adolescents with T1DM in Turkey.

### What this study adds?

The Turkish version of the Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes is valid and reliable scale in the assessment of diabetes resilience and strengths in adolescents with T1DM. Healthcare professionals can use this brief self-report scale to evaluate adaptive strengths and resilience related to diabetes management for adolescents with T1DM.

## Abstract

**Objective:** Resilience in diabetes refers to the capacity overcome diabetes-related challenges to achieve favorable psychosocial and health outcomes. Despite the known benefits of resilience in adolescents with type 1 diabetes mellitus (T1DM), there tends to be more emphasis on risk factors in research and practice. This study evaluated the psychometric properties of the Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes (DSTAR-Teen) in Turkey.

**Methods:** This descriptive, methodological study was conducted between October 2020 and May 2021. The Turkish DSTAR-Teen was administered to 120 adolescents with T1DM, and the data were evaluated using Cronbach's alpha coefficients, factor analyses, test-retest correlation, and item-total score correlations.

**Results:** The Turkish DSTAR-Teen has 12 items in two factors that explained 50.64% of the total variance. Confirmatory factor analysis revealed goodness-of-fit and comparative fit indices of 0.92 and 0.95, respectively. The total Cronbach's alpha value of the scale was 0.85. Item-total score correlations ranged from 0.49 to 0.74 ($p < 0.001$).

**Conclusion:** Our analyses showed that the Turkish DSTAR-Teen is a valid and reliable instrument in Turkish adolescents with T1DM. The Turkish DSTAR-Teen can be used to evaluate strengths and resilience associated with diabetes management in adolescents with T1DM in Turkey.

**Keywords:** Adolescent, diabetes, resilience, reliability, validity

## Introduction

Diabetes mellitus type 1 (T1DM) is a chronic metabolic disorder primarily caused by absolute insulin deficiency (1,2). T1DM is common among adolescents, and its incidence is increasing rapidly worldwide. The International Diabetes Federation (IDF) reported that in 2021, over 1.2 million children and adolescents had T1DM globally, 26,832 of which were in Turkey (3). According to the Ninth

**Address for Correspondence:** Burcu Aykanat Girgin PhD, Associate Professor, University of Health Sciences Turkey, Hamidiye Faculty of Nursing, Department of Pediatric Nursing, İstanbul, Turkey
**E-mail:** aykanat_87@hotmail.com **ORCID:** orcid.org/0000-0002-2601-8781

**Conflict of interest:** None declared
**Received:** 09.03.2022
**Accepted:** 23.05.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

edition of the IDF Diabetes Atlas, Turkey will be among the 10 countries with the largest population of children and adolescents with T1DM by 2035 (4).

The self-care tasks involved in diabetes management can be challenging for adolescents, who are already dealing with various psychological and physiological changes (2,5,6). These tasks include adhering to diet and exercise, measuring blood glucose, administering insulin, and responding to changes in blood glucose levels (2). During adolescence, the increased need for insulin, greater desire for independence, immature cognitive function, and more time spent out of the house can disrupt these routines and potentially lead to unwanted complications (7,8). Moreover, adolescents' changing relationship with their parents during this period, which is often marked by increased conflict, and the transfer of responsibility for T1DM management from parents to adolescents make it more difficult for parents to be involved in the process and reduces treatment adherence (9,10,11). Adolescents with T1DM are also susceptible to mental health disorders, such as diabetes distress and depression (12).

Diabetes resilience has been described as the ability to achieve favorable health and psychosocial outcomes in spite of the numerous challenges involved in living with and managing diabetes in adolescence (13). In adolescents with T1DM, diabetes resilience is reported to reduce the negative emotions associated with diabetes, increase self-efficacy behaviors in diabetes management, and facilitate metabolic control (14,15,16,17). Therefore, it is important to know and support the strengths and protective factors that increase resilience in patients with T1DM (14,18). In the literature, collaborative parental involvement, supportive family communication, problem-solving skills, and diabetes self-efficacy have been identified as strengths that contribute to resilience in the face of diabetes-related challenges (18,19,20). Despite the known advantages of resilience, the assessment and promotion of resilience often takes a back seat to the evaluation of problems and obstacles related to diabetes management in pediatric research and practice (14).

Although some resilience measures are available in the literature, they have various limitations, such as focusing on only one construct or the technical aspects of diabetes management and failing to reflect the adaptive nature of diabetes management in adolescents (18). As a result, Hilliard et al. (14) developed the Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes (DSTAR-Teen) to assess young people's self-efficacy and help-seeking behaviors in diabetes management. The DSTAR-Teen is a brief self-report tool for evaluating diabetes-specific strengths and resilience that can be easily filled out by adolescents and has been validated for use in this population. At present, there is no validated assessment tool to measure diabetes-specific resilience and strengths of adolescents with T1DM in the Turkish language. Concepts and manifestations of resilience may vary according to culture and environment (21,22). Therefore, the aim of this study was to adapt the DSTAR-Teen into Turkish and investigate its psychometric properties in adolescents with T1DM living in Turkey.

## Methods

### Participants

This descriptive, methodological study was conducted with 120 adolescents aged 14 to 18 years who were registered in the pediatric endocrinology outpatient and inpatient clinics of two training and research hospitals in western Turkey between October 2020 and May 2021. For validity and reliability studies, a sample size 5-10 times (23) or 10-20 times the number of items in the assessment tool is recommended (24). Sample sizes for analyzing the validity and reliability of a scale have also been categorized as good (100-500), very good (500-1000), and excellent (≥1000) (24,25). Based on this information, we included 120 adolescents who met the selection criteria in the sample for this study. For test-retest analysis, we administered the Turkish version of the DSTAR-Teen again, after a 3-week interval, to 24 of the adolescents in the sample (25).

Inclusion criteria were: 1) being between 14 and 18 years of age; 2) having a diagnosis of T1DM for at least 1 year; 3) receiving at least 0.5 units of insulin per kg per day; and 4) agreeing to participate and providing both personal and parental informed consent. Exclusion criteria were: 1) undergoing treatment for major depressive disorder or using antidepressant medication; and 2) having any hearing, speech, or cognitive impairment.

Written permission to carry out the Turkish adaptation and validity/reliability analyses of the DSTAR-Teen was obtained via e-mail from the developer of the original scale (14). Approval was obtained from the institutional review board of a Zeynep Kamil Maternity and Children Diseases Training and Research Hospital in İstanbul, Turkey (no: 08.07.2020/146). Institutional permissions were also obtained from the hospitals where data collection was carried out. Adolescents and their parents were met before initiating the study to inform them of the research purpose and procedures and their verbal and written consent was obtained.

### Instruments

**Descriptive questionnaire:** The adolescents answered nine questions (seven multiple choice and two open-ended) about their age, gender, their parents' education level, diabetes duration, frequency of blood glucose measurement, hemoglobin A1c (HbA1c) level, and instances of hypoglycemia and hyperglycemia in the last month.

**DSTAR-Teen:** Hilliard et al. (14) (2017) developed this scale to measure adolescents' self-efficacy and adaptive and help-seeking behaviors associated with diabetes management. The original scale comprises 12 items rated on a 5-point Likert-type scale (never = 1, almost always = 5). The DSTAR-Teen has a two-factor structure (diabetes-related confidence and help with diabetes management) that measures intrapersonal and interpersonal resilience and strengths, respectively. The six items in the diabetes-related confidence subscale assess adolescents' self-efficacy in managing their diabetes overall (item 1), dealing with abnormal blood glucose levels (item 3), asking questions to healthcare professionals (item 4), solving diabetes-related problems (item 5), and prioritizing diabetes management (item 10). The other item asks whether they believe their effort toward diabetes management makes a difference (item 8). The help with diabetes management subscale evaluates adolescents' comfort with telling their peers about their diabetes (item 2), their ability to ask for help from peers and parents (items 6, 7, 9, and 11), and their ability to communicate about diabetes with their parents (item 12).

Total scores on the DSTAR-Teen range from 12 to 60 points, and there is no defined cut-off point. A higher score represents greater resilience and strengths. The original scale had a Cronbach's alpha of 0.89 and item-total correlations between 0.55 and 0.78. Assessment of criterion validity demonstrated a significant association between adolescents' HbA1c values and their DSTAR-Teen subscale and total scores, with the adolescents who scored higher on the DSTAR-Teen having significantly more normal HbA1c values. The DSTAR-Teen was reported to be valid and reliable for the evaluation of adaptive behaviors and attitudes related to diabetes management in adolescents aged 14-18 years (14).

### Procedure

To ensure cross-cultural consistency and analyze the validity and reliability of the scale before data collection, we followed a 4-step process consisting of forward and back translation, expert opinion, and pilot testing, as recommended in guidelines and the Consensus-Based Standards for the Selection of Health Measurement Instruments standards (26,27,28).

### Forward and Back Translation

Two native Turkish-speaking linguists fluent in English and familiar with both cultures independently translated the scale into Turkish. We compared the translations and selected the most appropriate expressions to create a single form, which was then revised by a Turkish language expert. Two native English-speaking translators with knowledge of Turkish language and culture and experience in health terminology then independently translated the draft Turkish version of the DSTAR-Teen back into English. Their versions were compared with the original to ensure consistency in meaning.

### Expert Opinion

To evaluate content validity, we presented the original and Turkish versions of the scale to a panel of 10 experts including eight faculty members, one pediatric endocrinology and metabolism specialist, and one pediatric diabetes nurse (25,29). The experts assessed each item as very appropriate (1), appropriate (2), needs minor modification (3), and requires major revision (4) (30). Item-level and scale-level content validity indices (I-CVI/S-CVI) were calculated as described previously (31,32). Items were revised as needed based on the experts' feedback and the final version was used for pilot testing (25).

### Pilot Test

The scale was administered to 10 adolescents who volunteered to participate in the study during the enrollment phase but were not included in the sample (23). No negative feedback about the comprehensibility and readability of the scale items or the scale response time was received in the pilot test. Therefore, we concluded that the scale was sufficiently comprehensible, and the final version of the Turkish DSTAR-Teen (Appendix 1) was administered to the entire sample.

### Data Collection

Before data collection, the adolescents and their parents were informed about the purpose of the study, and written consent was obtained from those who agreed to participate. After obtaining informed consent, the participating adolescents were asked to complete the descriptive information form and Turkish version of DSTAR-Teen individually (Appendix 1). In addition to their self-report on the descriptive information form, the participants' most recent HbA1c value was checked from their outpatient and ward records. In case of a discrepancy, the value on record was accepted as correct. Completion of the data collection forms took approximately 15

minutes. Participants filled out the data collection forms at a convenient time of their choosing. Participation in the study was completely voluntary, and participants received no compensation.

### Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences Statistics, version 22.0 (IBM Corp, Armonk, NY) and AMOS software packages (https://www.ibm.com/products/structural-equation-modeling-sem). Sociodemographic information was summarized using number, percentage, minimum, maximum, and mean values.

Validity of the Turkish version of DSTAR-Teen was assessed through content, construct, and criterion validity analyses. In content validity analysis, CVI values were used to evaluate consistency in expert opinion (29). Construct validity was examined through exploratory factor analysis (EFA) and confirmatory factor analysis (CFA). EFA was performed using the principal components method with varimax rotation after evaluating the adequacy of the data with Bartlett's test of sphericity and the Kaiser-Meyer-Olkin (KMO) test (24,25,31). A p value $< 0.05$ in Bartlett's chi-square test was sought. KMO values were considered inappropriate below 0.50 and excellent as they approached 1 (25,31). CFA was done to verify that the structure of the Turkish version of the scale was consistent with that of the original. We performed CFA using goodness-of-fit index (GFI), comparative fit index (CFI), normal fit index (NFI), incremental fit index (IFI), root mean square error of approximation (RMSEA), and chi-square/degrees of freedom ($\chi^2$/df) ratio as model fit indices (23,33). For criterion validity, we evaluated the relationship between the adolescents' most recent HbA1c values and their total and subscale scores on the DSTAR-Teen Turkish version using Pearson correlation analysis to test the hypothesis that these parameters would be inversely associated (14,34).

Cronbach's alpha, item-total correlation (Pearson correlation test), and test-retest (paired samples t-test) analyses were used to assess the reliability of the Turkish DSTAR-Teen. Statistical significance was accepted at $p < 0.05$ (23,25).

## Results

Table 1 presents the sociodemographic and clinical characteristics of the adolescents included in the study. Their mean age was $15.23 \pm 1.31$ years and 50.2% were boys. Duration of T1DM was 1-3 years for 37.5% (n = 45) of the participants. The frequency of blood glucose measurement ranged from 2 to 12 times a day, with 31.7% (n = 38) measuring blood glucose 4-5 times a day and 25.8% (n = 31) measuring blood glucose 6-7 times a day. Evaluation of hypo/hyperglycemia frequency showed that 37.5% (n = 45) of participants reported having experienced hypoglycemia 1-3 times and 27.5% (n = 33) experienced hyperglycemia 1-3 times in the last month. The mean HbA1c value of the participants was $8.95 \pm 1.88$% (range: 4.7-15.3). This was higher than the recommended level of ≤7.5% for T1DM management.

### Validity Analysis

**Content validity:** Based on the 10 experts' feedback, I-CVI and S-CVI values for the Turkish version of DSTAR-Teen were 0.92 and 0.96, respectively.

**Construct validity (EFA and CFA):** The Turkish DSTAR-Teen had a KMO coefficient of 0.82, while Bartlett's test of sphericity $\chi^2$ value was 476.559 ($p = 0.001$). In EFA, the Turkish version of DSTAR-Teen showed a two-factor structure that explained 50.6% of the total variance. Item factor loadings varied between 0.620 and 0.778 (Table 2). In CFA, factor loading value ranges were 0.50 to 0.80 for the whole scale, 0.52 to 0.77 for the diabetes-related confidence subscale, and 0.50 to 0.80 for the help with diabetes management subscale (Figure 1). The model $\chi^2$ was 90.28, degrees of freedom (df) value was 53, and RMSEA was 0.077. The $\chi^2$/df ratio was 1.703. Other model fit index values were GFI = 0.92, CFI = 0.95, NFI = 0.96, and IFI = 0.95 (Table 3).

**Criterion validity:** Significant and strong negative correlations were observed between the participants' HbA1c values and their Turkish DSTAR-Teen total scores ($r = -0.947$; $p = 0.001$), diabetes-related confidence subscale scores ($r = -0.768$; $p = 0.001$), and help with diabetes management subscale scores ($r = -0.871$; $p = 0.001$). As DSTAR-Teen scores increased, the participants' HbA1c values decreased to within the reference range. These results supported the previously observed relationship between diabetes resilience and DSTAR-Teen scores.

### Reliability Analysis

Cronbach's alpha values for the diabetes confidence and help in diabetes management subscales were 0.80 and 0.81, respectively, and that of the entire scale was 0.85. Item-total score correlation coefficients ranged from 0.49 to 0.74 (Table 4; $p = 0.001$). Item test and retest scores showed no significant differences in the paired samples t-test, and test-retest correlation coefficients for the items ranged from 0.614 to 0.942 ($p = 0.001$; Table 5). The intraclass correlation coefficient was 0.91, indicating high test-retest reliability.

## Discussion

It has been reported that resilience in adolescents with T1DM reduces negative emotions associated with diabetes and facilitates the maintenance of metabolic control (14,16,17). Therefore, measures to evaluate and enhance resilience in patients with T1DM are important (14). In the first stage of our study, we reviewed the literature for strengths and resilience scales that have been developed for adolescents with T1DM. In this study, we adapted the DSTAR-Teen into Turkish and investigated its psychometric properties in the Turkish adolescent population.

### Validity Analysis

In this study we followed a 4-step process consisting of forward translation, back translation, expert opinion, and pilot testing to ensure language and content validity of the Turkish DSTAR-Teen (26,27,28). Based on evaluation by a panel of 10 experts, both the I-CVI (0.92) and S-CVI (0.96) of the Turkish DSTAR-Teen were well above the accepted threshold of 0.80 (31,35). Therefore, we concluded the items of the DSTAR-Teen were appropriate for Turkish culture and adequately represented the construct being measured (32).

We used the KMO coefficient and Bartlett's chi-square tests to evaluate whether the data were adequate and suitable

**Table 1. Descriptive characteristics of the adolescents with T1DM (n = 120)**

| Descriptive characteristics | | | |
|---|---|---|---|
| | | **Min-max (median)** | **Mean ± SD** |
| **Age (years)** | | 14-18 (15) | 15.23 ± 1.31 |
| **HbA1c (%)** | | 4.7-15.3 (8.2) | 8.95 ± 1.88 |
| | | **n** | **%** |
| **Gender** | Female | 59 | 49.2 |
| | Male | 61 | 50.8 |
| **Paternal education level** | Illiterate | 6 | 5.0 |
| | Primary school | 39 | 32.5 |
| | Secondary school | 53 | 44.2 |
| | University | 22 | 18.3 |
| **Maternal education level** | Illiterate | 7 | 5.8 |
| | Primary school | 60 | 50.0 |
| | Secondary school | 33 | 27.5 |
| | University | 20 | 16.7 |
| **Diabetes duration (years)** | 1-3 | 45 | 37.5 |
| | 4-6 | 31 | 25.8 |
| | 7-9 | 22 | 18.4 |
| | 10-12 | 16 | 13.3 |
| | 13-15 | 6 | 5.0 |
| **Daily frequency of blood glucose measurement (times)** | 2-3 times | 23 | 19.2 |
| | 4-5 times | 38 | 31.7 |
| | 6-7 times | 31 | 25.8 |
| | ≥8 times | 28 | 23.3 |
| **Frequency of hypoglycemia in last month (times)** | 1-3 times | 45 | 37.5 |
| | 4-6 times | 24 | 20.0 |
| | 7-9 times | 17 | 14.2 |
| | ≥10 times | 15 | 12.5 |
| | None | 19 | 15.8 |
| **Frequency of hyperglycemia in last month (times)** | 1-3 times | 33 | 27.5 |
| | 4-6 times | 30 | 25.0 |
| | 7-9 times | 21 | 17.5 |
| | ≥10 times | 27 | 22.5 |
| | None | 9 | 7.5 |

T1DM: type 1 diabetes mellitus, Min-max: minimum-maximum, HbA1c: hemoglobin A1c, SD: standard deviation

to conduct factor analysis. A KMO coefficient >0.50 and statistically significant result in Bartlett's chi-square test are recommended in order to perform EFA (33,35). In our study, we determined the Turkish DSTAR-Teen had a KMO of 0.82 and Bartlett's chi-square test was significant ($\chi^2 = 476.559$; p = 0.001), indicating that the scale and sample size were sufficient for factor analysis (35). These values were consistent with the Chinese version of the scale (34).

We found that the Turkish DSTAR-Teen conformed to a two-factor structure that explained 50.64 % of the total variance in the scale. The explained variance was above the expected values of >30 % for single-factor scales and >40 % for multi-factor scales (33,35), supporting its construct validity (23,33). While the Turkish version of the scale showed a two-factor structure consistent with the original (14), the Chinese version showed a three-factor structure. The authors noted that this may be a result of different cultures and environments (34).

The factor loading value is a coefficient that explains the relationships between the items and the factors of an assessment tool. In the literature, a factor loading of >0.30 (35,36) or >0.40 is recommended for an item to be included in a scale (23,30). The factor loadings for the Turkish DSTAR-Teen ranged between 0.620 and 0.778. As these values

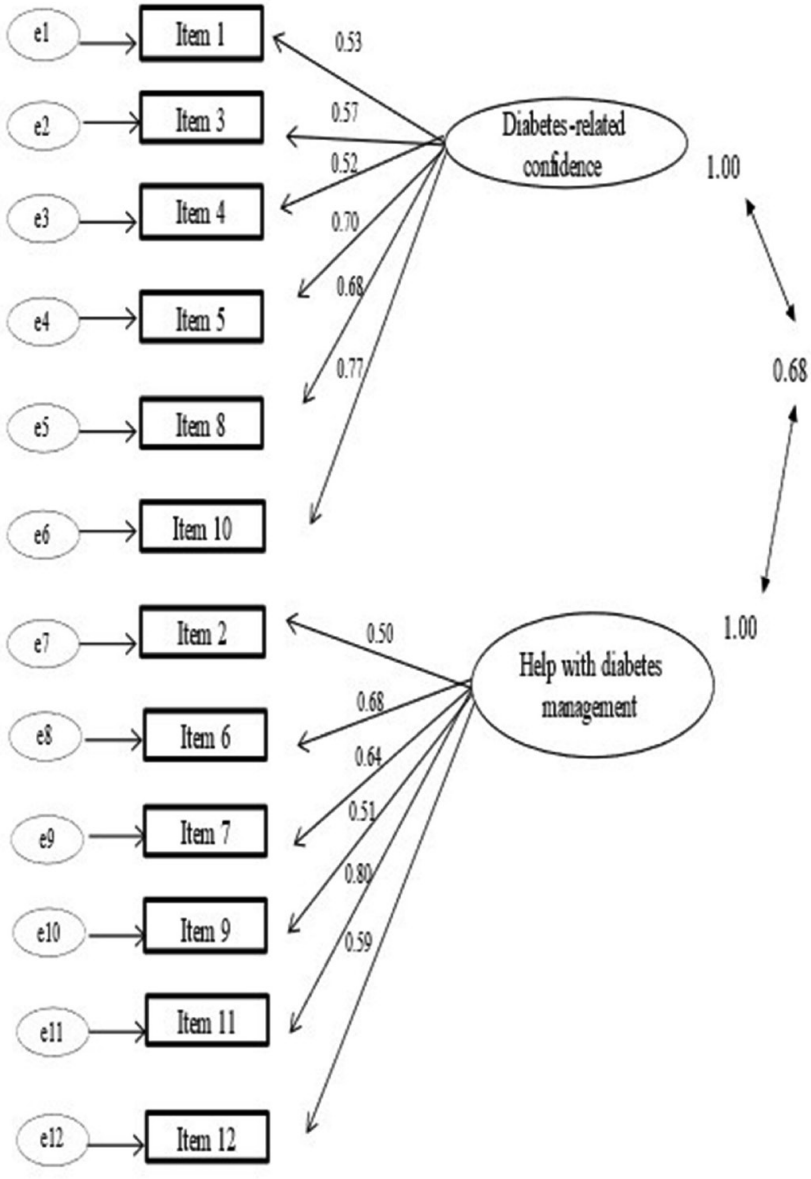


**Figure 1.** Factor structure of the Turkish version of DSTAR-Teen (p value for all factor loadings <0.001)

*DSTAR-Teen: Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes*

were greater than the threshold of 0.30, none of the items were removed (35). Factor loadings were reported as 0.50 to 0.93 in the original study of the scale (14) and between 0.59 and 0.72 in the Chinese version (34), similar to our study.

Confirmatory factor analysis is a method used when adapting a scale to a different language and culture than those for which it was developed. CFA examines the suitability of the factor structure determined by EFA (23,36). According to CFA analysis, item factor loadings were greater than 0.30 (Figure 1), RMSEA was less than 0.08, $\chi^2$/df was less than 5, and other model fit indices were greater than 0.90 (Table 3). The results of CFA were consistent with the two-factor structure of the Turkish DSTAR-Teen. The scale items were found to be relevant to their subscales and appropriate for evaluating the resilience and strengths of adolescents with T1DM in a Turkish sample (25,36). In the Chinese version of the scale, fit indices were reported as $\chi^2$/df = 2.29, GFI = 0.87, and CFI = 0.90 (34). GFI indices for the Turkish DSTAR-Teen were higher than those of the Chinese version. The results of EFA and CFA analysis demonstrated that the Turkish DSTAR-Teen had strong construct validity.

The relationship between adolescents' HbA1c values and DSTAR-Teen scores was evaluated in the original DSTAR-Teen study (14). The authors reported that adolescents with HbA1c > 7.5% had significantly higher total DSTAR-Teen scores (52.7 ± 6.2) than those with HbA1c < 7.5% (48.2 ± 8.0) and that HbA1c values were negatively correlated with DSTAR-Teen total score. Similarly, in the study of the Chinese version, a significant negative association was observed between DSTAR-Teen and HbA1c values (r = -0.21, p = 0.002) (34). As an assessment of criterion validity, we also tested the hypothesis that HbA1c values would decrease as scores on the Turkish DSTAR-Teen increased. We detected a significant negative correlation between the participants' HbA1c measurements and their total diabetes resilience scores (r = -0.947; p = 0.001), thus supporting the criterion validity of the scale.

## Reliability Analysis

The reliability of an instrument is defined as its correct measurement of the construct of interest and consistency in responses between individuals (23,37). Cronbach's alpha coefficient, a parameter of internal consistency reliability, reflects the consistency and homogeneity of the items in an assessment tool (23,25). In the literature, a minimum Cronbach's alpha of 0.70 is recommended for an assessment tool to be considered reliable in terms of internal consistency (25). An instrument is regarded as having good reliability at Cronbach's alpha coefficients between 0.70 and 0.80 and high reliability at values of 0.80 to 1 (25,37). The Cronbach's alpha coefficients for the Turkish DSTAR-Teen and its subscales were all greater than 0.80 in this study, indicating high reliability (Table 4).

**Table 2. Factor analysis of the Turkish version of DSTAR-Teen (n = 120)**

| Factor 1: Diabetes-related confidence | | Factor 2: Help with diabetes management | |
|---|---|---|---|
| **Item** | **Item factor loading** | **Item** | **Item factor loading** |
| 1. I am able to take care of my diabetes pretty well. | 0.666 | 2. I tell my friends about diabetes. | 0.647 |
| 3. I am good at responding to high or low blood sugar. | 0.620 | 6. My parent(s) help me take care of my diabetes. | 0.656 |
| 4. I am able to ask my nurse or doctor questions about how to manage my diabetes. | 0.647 | 7. I can ask for help with my diabetes management when I need to. | 0.632 |
| 5. I am good at figuring out what to do for my diabetes care when problems come up. | 0.715 | 9. I can count on my friends to help me take care of diabetes if I need help. | 0.714 |
| 8. If I try hard to do everything I need to do for my diabetes, it makes a difference. | 0.621 | 11. There is someone I can always ask for help with my diabetes. | 0.716 |
| 10. I can figure out ways to take care of my diabetes even when I am busy or other things make diabetes hard to manage | 0.778 | 12. I talk to my parent(s) calmly about diabetes, like talking about my A1c or remembering to do blood sugar checks. | 0.655 |
| **Eigen value** | 3.090 | | 2.986 |
| **Variance (%)** | 25.751 | | 24.887 |
| **Total explained variance (%)** | 50.638 | | |

DSTAR-Teen: Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes

**Table 3. Model fit indices for confirmatory factor analysis**

| Model | $\chi^2$ | df[a] | $\chi^2$/df | RMSEA | p | GFI | CFI | NFI | IFI |
|---|---|---|---|---|---|---|---|---|---|
| Two-factor | 90.28 | 53 | 1.70 | 0.077 | < 0.001 | 0.92 | 0.95 | 0.96 | 0.95 |

[a]Degree of freedom.
RMSEA: Root mean square error of approximation, GFI: goodness of fit index, CFI: comparative fit index, NFI: normed fit index, IFI: incremental fit index

These findings provide evidence that the Turkish version of DSTAR-Teen and its subscales are consistent and relevant, show homogeneity, and that the items are reliable in the assessment of strengths and resilience in adolescents with T1DM. Similar to our study, Cronbach's alpha coefficients of 0.89 were reported for the original and Chinese versions of the scale (14,34).

**Table 4. Cronbach's alpha coefficient and item-total score correlation of the Turkish version of the DSTAR-Teen (n = 120)**

| Turkish version of DSTAR-Teen | Item-total score correlation | Mean ± SD |
|---|---|---|
| | r* | |
| I am able to take care of my diabetes pretty well | 0.49 | 4.01 ± 0.85 |
| I tell my friends about diabetes. | 0.55 | 3.98 ± 1.24 |
| I am good at responding to high or low blood sugar. | 0.60 | 3.88 ± 1.17 |
| I am able to ask my nurse or doctor questions about how to manage my diabetes. | 0.51 | 4.08 ± 0.98 |
| I am good at figuring out what to do for my diabetes care when problems come up. | 0.65 | 4.03 ± 1.03 |
| My parent(s) help me take care of my diabetes. | 0.60 | 4.63 ± 0.67 |
| I can ask for help with my diabetes management when I need to. | 0.68 | 4.15 ± 1.03 |
| If I try hard to do everything I need to do for my diabetes, it makes a difference. | 0.69 | 4.01 ± 1.09 |
| I can count on my friends to help me take care of diabetes if I need help. | 0.55 | 3.14 ± 1.51 |
| I can figure out ways to take care of my diabetes even when I am busy or other things make diabetes hard to manage. | 0.67 | 3.88 ± 0.98 |
| There is someone I can always ask for help with my diabetes. | 0.74 | 4.23 ± 1.11 |
| I talk to my parent(s) calmly about diabetes, like talking about my A1c or remembering to do blood sugar checks. | 0.60 | 4.16 ± 1.05 |
| Diabetes-related Confidence subscale | | 23.89 ± 4.30 |
| Help with Diabetes Management subscale | | 24.29 ± 4.63 |
| Total Turkish DSTAR-Teen | | 48.18 ± 7.74 |
| Cronbach's alpha | Scale/Subscale | Cronbach's alpha coefficient |
| | Diabetes-related confidence (items 1, 3, 4, 5, 8, 10) | 0.80 |
| | Help with diabetes management (items 2, 6, 7, 9, 11, 12) | 0.81 |
| | Total Turkish DSTAR-Teen | 0.85 |

*r: Pearson correlation analysis, $p < 0.001$.
DSTAR-Teen: Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes, SD: standard deviation

**Table 5. Test-retest values of Turkish version of DSTAR-Teen (n = 24)**

| Items | Test | Retest | Pearson correlation analysis | | Paired samples t-test | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | r* | p | t | p |
| Item 1 | 4.09 ± 0.68 | 4.14 ± 0.83 | 0.646 | 0.001** | -0.326 | 0.747 |
| Item 2 | 4.18 ± 1.10 | 4.36 ± 0.85 | 0.898 | 0.001** | -1.702 | 0.104 |
| Item 3 | 3.64 ± 1.14 | 3.77 ± 1.15 | 0.735 | 0.001** | -0.767 | 0.451 |
| Item 4 | 4.00 ± 1.02 | 4.18 ± 0.85 | 0.873 | 0.001** | -1.702 | 0.104 |
| Item 5 | 4.09 ± 0.87 | 4.18 ± 0.91 | 0.825 | 0.001** | -0.810 | 0.427 |
| Item 6 | 4.86 ± 0.35 | 4.77 ± 0.43 | 0.733 | 0.001** | 1.449 | 0.162 |
| Item 7 | 4.23 ± 0.92 | 4.27 ± 0.70 | 0.635 | 0.001** | -0.295 | 0.771 |
| Item 8 | 4.18 ± 0.73 | 4.18 ± 0.66 | 0.614 | 0.001** | 0.000 | 1.000 |
| Item 9 | 3.14 ± 1.55 | 3.23 ± 1.54 | 0.942 | 0.001** | -0.810 | 0.427 |
| Item 10 | 4.09 ± 0.75 | 4.18 ± 0.73 | 0.835 | 0.001** | -1.000 | 0.329 |
| Item 11 | 4.59 ± 0.80 | 4.55 ± 0.74 | 0.802 | 0.001** | 0.439 | 0.665 |
| Item 12 | 4.55 ± 0.60 | 4.45 ± 0.80 | 0.753 | 0.001** | 0.810 | 0.427 |

*r: Pearson correlation analysis, **$p < 0.01$.
DSTAR-Teen: Diabetes Strengths and Resilience Measure for Adolescents with Type 1 Diabetes, SD: standard deviation

Test-retest analysis determines whether an instrument shows consistency and stability between two assessments performed under the same conditions after a certain period of time. For test-retest analysis, the instrument should be administered to at least 20 people with an interval of two to six weeks (25). Therefore, we administered the Turkish version of the DSTAR-Teen again after a 3-week interval to 24 of the adolescents in the sample. The test-retest results of the Turkish DSTAR-Teen showed significant correlations ($p = 0.001$), with no statistical differences in mean item scores between the test and retest and an intraclass correlation coefficient of 0.91 (Table 5). These findings demonstrate that the Turkish scale was highly reliable and consistently measured the constructs of resilience and strengths at different times in adolescents with T1DM (23,25). Our results were consistent with those reported for the Chinese version of the scale (34). However, we could not compare our results with the findings reported by Hilliard et al. (14) (2017) because they did not conduct test-retest analysis.

Item-total score correlations demonstrate how scores obtained from individual items relate to the total scale score and to what extent the items differentiate, based on the measured characteristic (35,36). Item-total score correlation coefficients are expected to be positive and greater than 0.30 (23,24). In our study, item-total score correlations were between 0.49 and 0.74. Therefore, we concluded that all items were related to the scale, adequately measured resilience and strengths in adolescents with T1DM, and had high reliability (24,35). In the original study of the scale, item-total correlations were between 0.55 and 0.78, similar to our study (14). As item-total correlations were not examined for the Chinese version of the scale, no comparison could be made (34).

In our study, the mean total scale score of adolescents was $48.18 \pm 7.74$, which is above the average of the potential score range. Mean total scores were reported as $49.0 \pm 7.9$ for the original scale and 42.4 for the Chinese version. Our results are similar to the original study (14) and may be higher than in the Chinese study because they included adolescents diagnosed with major depression (34).

### Study Limitations

Because this study was conducted in two centers and used convenience sampling, our results may have limited representativeness and generalizability.

## Conclusion

The Turkish version of the DSTAR-Teen has 12 items in two subscales, consistent with the original, and showed a high Cronbach's alpha. The results of our analyses demonstrate that the Turkish DSTAR-Teen is valid and reliable in the assessment of diabetes resilience and strengths in adolescents in Turkey. All healthcare professionals can use this brief self-report scale to evaluate adaptive strengths and resilience related to diabetes management and investigate their effects on glycemic control and mental health in adolescents with T1DM. We also believe the DSTAR-Teen will be useful in experimental studies evaluating the effect of interventions to improve resilience behaviors in adolescents with T1DM.

### Acknowledgements

We would like to thank the adolescents participating in the study.

### Ethics

**Ethics Committee Approval:** The study was approved by the Zeynep Kamil Maternity and Children Diseases Training and Research Hospital of Ethics Committee (protocol no: 146, date: 08.07.2020).

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

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Heves Kırmızıbekmez, Concept: Aslı Demirtaş, Burcu Aykanat Girgin, Design: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Data Collection or Processing: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Heves Kırmızıbekmez, Analysis or Interpretation: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Heves Kırmızıbekmez, Literature Search: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Heves Kırmızıbekmez, Writing: Aslı Demirtaş, Burcu Aykanat Girgin, Ayla Güven, Heves Kırmızıbekmez.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, Aschner P, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes 2018;19 Suppl 27(Suppl 27):7-19.
2. Ziegler R, Neu A. Diabetes in childhood and adolescence. Dtsch Arztebl Int 2018;115:146-156.
3. International Diabetes Federation (IDF). Diabetes Atlas, 10th ed. Brussels, 2021.
4. International Diabetes Federation (IDF). Diabetes Atlas, 9th ed. Brussels, 2019.

5. Holmström MR, Häggström M, Söderberg S. Being facilitators in a challenging context-school personnel's experiences of caring for youth with diabetes type 1. J Pediatr Nurs 2018;43:e114-e119. Epub 2018 Aug 10
6. Lohan A, Morawska A, Mitchell A. Associations between parental factors and child diabetes-management-related behaviors. J Dev Behav Pediatr 2017;38:330-338.
7. Markowitz JT, Garvey KC, Laffel LM. Developmental changes in the roles of patients and families in type 1 diabetes management. Curr Diabetes Rev 2015;11:231-238.
8. Wasserman RM, Hilliard ME, Schwartz DD, Anderson BJ. Practical strategies to enhance executive functioning and strengthen diabetes management across the lifespan. Curr Diab Rep 2015;15:52.
9. Hilliard ME, Wu YP, Rausch J, Dolan LM, Hood KK. Predictors of deteriorations in diabetes management and control in adolescents with type 1 diabetes. J Adolesc Health 2013;52:28-34. Epub 2012 Jul 15
10. Tremolada M, Cusinato M, Bonichini S, Fabris A, Gabrielli C, Moretti C. Health-related quality of life, family conflicts and fear of injecting: perceptions differences between preadolescents and adolescents with type 1 diabetes and their mothers. Behav Sci 2021;11:98.
11. Wiebe DJ, Chow CM, Palmer DL, Butner J, Butler JM, Osborn P, Berg CA. Developmental processes associated with longitudinal declines in parental responsibility and adherence to type 1 diabetes management across adolescence. J Pediatr Psychol 2014;39:532-541. Epub 2014 Mar 6
12. Buchberger B, Huppertz H, Krabbe L, Lux B, Mattivi JT, Siafarikas A. Symptoms of depression and anxiety in youth with type 1 diabetes: A systematic review and meta-analysis. Psychoneuroendocrinology 2016;70:70-84. Epub 2016 Apr 29.
13. Hilliard ME, Harris MA, Weissberg-Benchell J. Diabetes resilience: a model of risk and protection in type 1 diabetes. Curr Diab Rep 2012;12:739-748.
14. Hilliard ME, Iturralde E, Weissberg-Benchell J, Hood KK. Strengths and Resilience Measure for Adolescents with Type 1 Diabetes (DSTAR-Teen): validation of a new, brief self-report measure. J Pediatr Psychol 2017;42:995-1005.
15. Santos FR, Bernardo V, Gabbay MA, Dib SA, Sigulem D. The impact of knowledge about diabetes, resilience and depression on glycemic control: a cross-sectional study among adolescents and young adults with type 1 diabetes. Diabetol Metab Syndr 2013;5:55.
16. Wang RH, Hsu HC, Kao CC, Yang YM, Lee YJ, Shin SJ. Associations of changes in psychosocial factors and their interactions with diabetes distress in patients with type 2 diabetes: a longitudinal study. J Adv Nurs 2017;73:1137-1146. Epub 2016 Nov 28
17. Yi-Frazier JP, Yaptangco M, Semano S, Buscaino E, Thompson V, Cochrane K, Tabile M, Alving E, Rosenberg AR. The association of personal resilience with stress, coping and diabetes outcomes in adolescents with type 1 diabetes: variable-and person-focused approaches. J Health Psychol 2015;20: 1196-1206. Epub 2013 Nov 21
18. Hilliard ME, McQuaid EL, Nabors L, Hood KK. Resilience in youth and families living with pediatric health and developmental conditions: introduction to the special issue on resilience. J Pediatr Psychol 2015;40:835-839. Epub 2015 Aug 14
19. Agustini N, Nurhaeni N, Pujasari H, Abidin E, Lestari AW, Kurniawati A. Family Support towards Resilience in Adolescents with Type I Diabetes: A Preliminary Study in Indonesia. Asian Pac Isl Nurs J 2019;4:66-71.
20. Jaser SS, White LE. Coping and resilience in adolescents with type 1 diabetes. Child Care Health Dev 2011;37:335-342. Epub 2010 Dec 9
21. Ungar M. The social ecology of resilience: addressing contextual and cultural ambiguity of a nascent construct. Am J Orthopsychiatry 2011;81:1-17.
22. Ye ZJ, Qiu HZ, Li PF, Chen P, Liang MZ, Liu ML, Yu YL, Wang SN, Quan XM. Validation and application of the Chinese version of the 10-item Connor-Davidson Resilience Scale (CD-RISC-10) among parents of children with cancer diagnosis. Eur J Oncol Nurs 2017;27:36-44. Epub 2017 Feb 12
23. Şencan H. Reliability and Validity in Social and Behavioral Measurements. Ankara, Seçkin Publishing, 2005;89-100.
24. Şimşek ÖF. Introduction to Structural Equation Modeling, LISREL Fundamental Principles and Practices. İstanbul, Ekinoks Publishing, 2010;37-60.
25. Karagöz Y. SPSS and AMOS 23 Applied Statistical Analysis. Ankara, Nobel Publishing, 2018;50-160.
26. International Test Commission. ITC guidelines for translating and adapting tests. Last Accessed Date: 12.09.2020. Available from: https://www.intestcom.org/files/guideline_test_adaptation_2ed.pdf
27. World Health Organization. Process of translation and adaptation of instruments. Last Accessed Date: 06.02.2020. Available from: https://www.mhinnovation.net/sites/default/files/files/WHO%20Guidelines%20on%20Translation%20and%20Adaptation%20of%20Instruments.docx
28. Mokkink LB, Terwee CB, Patrick DL, Alonso J, Stratford PW, Knol DL, Bouter LM, de Vet HC. The COSMIN checklist for assessing the methodological quality of studies on measurement properties of health status measurement instruments: an international Delphi study. Qual Life Res 2010;19:539-549. Epub 2010 Feb 19
29. Polit DF, Beck CT, Owen SV. Is the CVI an acceptable indicator of content validity? Appraisal and recommendations. Res Nurs Health 2007;30:459-467.
30. Çam MO, Baysan Arabacı L. Qualitative and quantitative steps on attitude scale construction. Journal of Research and Development in Nursing 2010;12:59-71.
31. Polit DF, Beck CT. Essentials of Nursing Research: Appraising Evidence for Nursing Practice. Philadelphia, Lippincott Williams & Wilkins, 2018;25-90.
32. Ruddock HK, Christiansen P, Halford JCG, Hardman CA. The development and validation of the addiction-like eating behaviour scale. Int J Obes (Lond) 2017;41:1710-1717. Epub 2017 Jul 5
33. Çokluk Ö, Şekercioğlu G, Büyüköztürk S. Multivariate SPSS and LISREL Applications for the Social Science. 5th ed. Ankara, Pegem Academy Publishing, 2018;89-100.
34. Xu J, Luo D, Zhu M, Wang H, Shi Y, Ya D, Lin Z, Gu Z. Translation and its psychometric characteristics of the diabetes strengths and resilience measure among Chinese adolescents with type 1 diabetes. J Pediatr Nurs 2020;50:e2-e7. Epub 2019 Sep 13
35. De Vellis RF. Scale Development Theory and Applications. 4th ed. London, SAGE Publication Inc, 2017;70-135.
36. Jonhson B, Christensen L. Educational Research: Quantitative, Qualitative, and Mixed Approaches. 5th ed. London, SAGE Publication, 2014.
37. Büyüköztürk Ş. Data Analysis Handbook for Social Sciences. Ankara, Pegem Academy Publishing, 2014;179-194.

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SHORT COMMUNICATION

DOI: 10.4274/jcrpe.galenos.2022.2022-12-9

J Clin Res Pediatr Endocrinol 2022;14(3):334-338

# Diagnostic Power of Bilateral Inferior Petrosal Sinus Sampling with Desmopressin in Paediatric Cushing's Disease

**Manuel André Virú-Loza[1], Andrea Venegas Quispe[2]**

*[1]Hospital Nacional Dos de Mayo, Lima, Peru*

*[2]ADIECS Association for the Development of Student Research in Health Sciences, Universidad Nacional Mayor de San Marcos, Lima, Peru*

**What is already known on this topic?**

Bilateral inferior petrosal sinus sampling (BIPSS) with corticotropin releasing hormone (CRH) stimulation is an accurate test to diagnose Cushing's disease (CD). However, the use of desmopressin has been suggested as an alternative to CRH.

**What this study adds?**

To the best of our knowledge, this is the first review of this topic. BIPSS with desmopressin stimulation is accurate for the diagnosis of pediatric CD, but the accuracy of lateralizing the lesion is probably not suitable for pediatric clinical practice.

## Abstract

**Objective:** The aim of this study was to evaluate the diagnostic accuracy of bilateral inferior petrosal sinus sampling (BIPSS) with desmopressin for pediatric Cushing's disease (CD).

**Methods:** We reviewed studies performed in children that evaluated the accuracy of BIPSS with desmopressin.

**Results:** All included studies were case series of children with adrenocorticotropin hormone (ACTH)-dependent Cushing's syndrome. The overall accuracy of BIPSS before stimulation was 84.1% (37/44), and after stimulation it was 92.3% (36/39). The overall lateralizing accuracy of BIPSS was 50.0%.

**Conclusion:** Considering that available evidence is limited, it appears that BIPSS with desmopressin stimulation is accurate for the diagnosis of pediatric CD, but its lateralizing accuracy is probably not suitable for pediatric clinical practice.

**Keywords:** Petrosal sinus sampling, deamino arginine vasopressin, child, pituitary ACTH hypersecretion, systematic review

## Introduction

Cushing's syndrome (CS) occurs from prolonged exposure to high levels of cortisol and can be exogenous or endogenous (1). Endogenous CS has an annual incidence of approximately 0.7 to 3 cases per million people (1,2) and can be of two types: adrenocorticotropin hormone (ACTH)-dependent (pituitary tumor or ectopic ACTH syndrome) or ACTH-independent (autonomous adrenal overproduction of cortisol) (1). Overall, among patients with endogenous CS, 70% have a pituitary adenoma (3). ACTH-secreting pituitary adenoma is called Cushing's disease (CD) (1).

There are non-invasive tests to diagnose CD, such as ACTH measurement in the morning, the corticotropin releasing hormone (CRH) stimulation test, and the suppression test with high dose dexamethasone. (1). However, non-invasive tests are less accurate than bilateral inferior petrosal sinus sampling (BIPSS) (1). In a previous study, BIPSS correctly identified 139 out of 140 children with CD (4). Moreover, BIPSS is more accurate than images to locate a microadenoma, which it achieves by demonstrating the lateralization of ACTH secretion (2). It has been reported that the lateralization of ACTH in BIPSS correctly identifies the location of the adenoma in 70% of cases (4).

BIPSS consists of the placement of femoral catheters that reach the right and left inferior petrosal sinuses (5). After this, blood samples are obtained for measurement of ACTH from both petrosal sinuses and from a peripheral pathway before and after the administration of human CRH (5).

**Address for Correspondence:** Manuel André Virú-Loza MD, Hospital Nacional Dos de Mayo, Lima, Peru
**Phone:** +51 989281141 **E-mail:** m.andre.viru@gmail.com **ORCID:** orcid.org/0000-0001-6637-6463

**Conflict of interest:** None declared
**Received:** 03.01.2022
**Accepted:** 12.03.2022

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

However, the use of stimulation with desmopressin (DDAVP) has been suggested as an alternative to CRH (1).

A synthesis of the evidence on the performance of BIPSS with desmopressin stimulation in children is lacking. For this reason, the aim of this study was to evaluate the diagnostic accuracy of BIPSS with desmopressin for pediatric CD.

## Methods

### Population and Study Selection

The original protocol was registered in the PROSPERO database (6). We included studies performed in children (≤18 years) with CS (based on clinical features and/or laboratory results) that evaluated the accuracy of any of the following parameters: inferior petrosal sinus to peripheral ACTH ratio before stimulation with desmopressin; inferior petrosal sinus to peripheral ACTH ratio after stimulation with desmopressin at any time point or interpetrosal sinus gradient of one of the two sides to the contralateral side. The target condition was CD. The reference standard was histopathology. Within the inclusion criteria, those studies with the following study designs were considered: cross-sectional, case-control and cohort. In studies where only a subgroup of participants was eligible, these were included only if data specific to that subgroup could be extracted. The search was conducted up to February 14, 2021. There were no restrictions on language or publication date. We searched in the following electronic databases: PubMed, Embase, Scopus, and Web of Science. We also conducted a hand-search of reference lists of all included articles and relevant review articles to identify potentially eligible studies.

We used the following search strategies:

**PubMed:** (cushing*[tiab] OR "cushing syndrome"[mesh] OR "cushing syndrome"[ot]) AND ("petrosal sinus sampling"[mesh] OR "petrosal sinus"[tiab] OR "petrosal sinus sampling"[ot] OR BIPSS[tiab]) AND (child[mesh] OR child*[tiab] OR child[ot] OR paediatric*[tiab] OR pediatrics[mesh] OR pediatric*[tiab] OR pediatrics[ot] OR adolescent[mesh] OR adolescen*[tiab] OR adolescent[ot] OR youth*[tiab] OR teen*[tiab])

**Embase:** cushing* AND ('petrosal sinus'/exp OR 'petrosal sinus' OR bipss) AND (child* OR paediatric* OR pediatric* OR adolescen* OR youth* OR teen*)

**Scopus:** TITLE-ABS-KEY ((cushing*) AND ("petrosal sinus" OR BIPSS) AND (child* OR paediatric* OR pediatric* OR adolescen* OR youth* OR teen*))

**Web of Science:** TS = ((cushing*) AND ("petrosal sinus" OR BIPSS) AND (child* OR paediatric* OR pediatric* OR adolescen* OR youth* OR teen*))

We downloaded all articles from the electronic search to EndNote X8 and duplicate records were removed. All unique articles were uploaded to Rayyan (https://rayyan.qcri.org/) for the study selection process. Titles and abstracts were independently screened by two review authors to identify relevant studies. Likewise, two review authors independently examined the full text of selected studies and registered reasons for exclusion. Any disagreement on title/abstract and full-text selection was resolved by consensus.

### Data Extraction and Statistical Analysis

The information from each selected study was independently extracted by two review authors using a standardized data extraction form in an Excel spreadsheet that was previously piloted. Any disagreement was resolved by consensus. The following data was extracted: first author name, publication year, country, study design, sample size, population, age, sex, comorbidities, index tests, gold standard, adverse events, and the concordance rate of the three index tests. We calculated baseline inferior petrosal sinus to peripheral ACTH ratio, stimulated inferior petrosal sinus to peripheral ACTH ratio at any time point and interpetrosal sinus gradient of one of the two sides to the contralateral side as measures of accuracy. Data from selected studies were not suitable for performing further data synthesis techniques. As the included studies were only case series, no risk of bias assessment was performed.

Ethical approval was not required for this systematic review, as this study did not directly or indirectly involve human participants. Data were extracted from publicly available published literature.

## Results

We selected four studies published from 2007 to 2020 (7,8,9,10). Two of them were performed in Brazil (7,9) and the rest were conducted in China (8) and Argentina (10). All studies were case series of children with ACTH-dependent CS (7,8,9,10). None of the studies showed information about comorbidities (7,8,9,10). In two studies there was no information about adverse events available (8,10), one of them reported no complications (7) and the other described hematomas of the groin at the site of venous puncture in some patients, and two major complications even with the use of heparin infusion during the procedure: peripheral venous thrombosis in one patient with a previous thromboembolic event, and right petrosal sinus thrombosis

in another patient with severe hypercortisolism who finally died from sepsis and multiple-organ failure syndrome (9). More information about the selected studies can be found in Table 1.

The overall accuracy of BIPSS before stimulation was 84.1 % (37/44), and after stimulation it was 92.3 % (36/39) (7,8,9,10). The overall lateralizing accuracy of BIPSS was 50.0 % (19/38) (7,8,10). Cavalcante et al. (7) used stimulation with desmopressin and reported a concordance of 60 % (9/15). However, they did not provide complete data to assess accuracy before and after stimulation separately. In contrast, two studies performed BIPSS with desmopressin stimulation and reported an overall accuracy of 6/16 (8) and 4/7 (10). Furthermore, the same studies reported a joint accuracy of 39.1 % (9/23) and 38.9 % (7/18) before and after stimulation, respectively (8,10).

## Discussion

The overall accuracy of BIPSS before stimulation was good (84.1 %) (7,8,9,10), which is consistent with this test being considered the gold standard to differentiate pituitary from ectopic CS (1). Moreover, after stimulation accuracy was higher and more suitable for clinical practice (92.3 %) (7,8,9,10). Furthermore, this good performance was consistent with a recent study conducted in children using CRH stimulation that showed an unstimulated accuracy of 75 % (9/12) and a stimulated accuracy of 83.3 % (10/12) (5). Therefore, BIPSS with desmopressin stimulation in pediatric CD has a good diagnostic performance and is clinically more useful than when not using stimulation.

The overall lateralizing accuracy of BIPSS in the studies reviewed (50 %) (7,8,10) was not optimal for clinical practice. This is in accordance with some authors who state that the capacity of BIPSS to localize intra-pituitary tumors is limited, regardless of the stimulus used (1). Recent studies of BIPSS with CRH stimulation performed in children showed a lateralizing accuracy of 58.3 % (7/12) (5) and 69 % (9/13) (11). However, it was not specified whether the accuracy was calculated before or after stimulation. Other studies performed in pediatric and adult CD patients, with and without stimulation by CRH or desmopressin, also describe lateralization accuracy but they do not show separate information for children and do not detail the precision of BIPSS before and after the stimulus separately (12,13,14,15). Feng et al. (13) reported a concordance of 72.5 % (37/51) in patients who had CD and whose lateralization by BIPSS and surgery were either left or right. Wind et al. (15) reported a concordance of 68.9 % (273/396) in patients with pathologic confirmation of CD who underwent BIPSS and had a lateral adenoma. Pereira et al. (14) reported a lateralization accuracy of 63 % (17/27) in patients who had confirmation of CD (immunohistochemistry to ACTH or biochemical cure

**Table 1. Detailed information on the studies selected for this review**

| First author and year of publication | Sample size | Population | Age (years) | Sex | Index tests | Gold standard | Accuracy | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | bIP/P | sIP/P | IPg |
| Cavalcante et al. (7), 2020 | 19 (15 had CD and were submitted to BIPSS before surgery). | Patients with ACS submitted to BIPSS. | 9-19 | 9 F and 9 M | bIPS/P > 2<br>sIPS/P > 3<br>IPSg > 1.4 | Surgical and pathologic findings, postsurgical remission, Nelson's syndrome after bilateral adrenalectomy or an absence of EAS in the follow-up. | 14/15 | 15/15 | 9/15 * |
| Chen et al. (8), 2019 | 16 | Patients with CD submitted to BIPSS. | 9.8-18.7 | 10 F and 6 M (5 F and 6 M for those who received DDAVP) | bIPS/P > 2<br>sIPS/P > 3<br>IPSg > 1.4 | Surgical and pathologic findings or eucortisolism after surgery or irradiation. | 11/16 | 10/11 | 6/16 (basal: 5/16; stimulated: 3/11) |
| Gil et al. (10), 2019 | 7 | Patients with CD submitted to BIPSS. | 8 - 13 | 4 F and 3 M. | bIPS/P≥2<br>sIPS/P≥3<br>IPSg≥1.4 | Surgical and pathologic findings or clinical outcome. | 7/7 | 6/7 | 4/7 (basal and stimulated) |
| Machado et al. (9), 2007 | 6 (5 had CD and 1 had EAS) | Patients with ACS submitted to BIPSS. | 14 - 17 | 4 F and 2 M | bIPS/P≥2<br>sIPS/P≥3<br>IPSg≥1.4 | Pathologic findings or postsurgical remission | 5/6 | 5/6 | NA |

*Incomplete data to evaluate concordance before and after stimulation separately.
F: females, M: males, ACTH: adrenocorticotropin hormone, bIP/P: baseline inferior petrosal sinus to peripheral ACTH ratio, sIP/P: stimulated inferior petrosal sinus to peripheral ACTH ratio at any time point, IPg: interpetrosal sinus gradient of one of the two sides to the contralateral side, ACS: ACTH dependent CS, EAS: ectopic ACTH syndrome, NA: information not available

criteria after surgery) and a BIPSS suggestive of CD. Deipolyi et al. (12) reported a lateralization accuracy of 47.8% (87/182) in patients with pathologic confirmation of CD. Of note, there was considerable variability in the lateralizing accuracy reported in individual studies, ranging from 38.9% to 100% (7,8,10,16,17). Therefore, there is also a need for more evidence concerning this topic.

Among the reasons reported as potential causes of false negatives are incorrect cannulation of the inferior petrosal sinuses due to anatomical variants or the performance of the procedure during a phase of eucortisolism (1).

Of the patients included in this review, only two had major complications (peripheral venous thrombosis and right petrosal sinus thrombosis) (9). This coincides with some authors who report that there is a low incidence of complications in experienced centers. However, when complications do occur they can be severe, such as deep vein thrombosis, petrosal sinus thrombosis, and hemorrhage (1).

### Study Limitations

An important limitation of this review is the limited number of published studies and hence small amount of evidence available. This probably affects the interpretation of the data on the lateralizing accuracy of BIPSS to a greater degree than the diagnostic accuracy of the test.

## Conclusion

In conclusion, considering that available evidence is limited, we suggest that BIPSS with desmopressin stimulation is accurate for the diagnosis of pediatric CD, but its lateralizing accuracy is probably not suitable for pediatric clinical practice. Also, there is not enough evidence to confirm the safety of this test in children.

### Ethics

**Ethics Committee Approval and Informed Consent:** Ethical approval was not required for this systematic review, as this study did not directly or indirectly involve human participants.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Manuel André Virú-Loza, Concept: Manuel André Virú-Loza, Andrea Venegas Quispe, Design: Manuel André Virú-Loza, Andrea Venegas Quispe, Data Collection or Processing: Manuel André Virú-Loza, Andrea Venegas Quispe, Analysis or Interpretation: Manuel André Virú-Loza, Andrea Venegas Quispe, Literature Search: Manuel André Virú-Loza, Andrea Venegas Quispe, Writing: Manuel André Virú-Loza, Andrea Venegas Quispe.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Barbot M, Zilio M, Scaroni C. Cushing's syndrome: Overview of clinical presentation, diagnostic tools and complications. Best Pract Res Clin Endocrinol Metab 2020;34:101380. Epub 2020 Jan 30
2. Ferrigno R, Hasenmajer V, Caiulo S, Minnetti M, Mazzotta P, Storr HL, Isidori AM, Grossman AB, De Martino MC, Savage MO. Paediatric Cushing's disease: Epidemiology, pathogenesis, clinical management and outcome. Rev Endocr Metab Disord 2021;22:817-835. Epub 2021 Jan 30
3. Miller BS, Auchus RJ. Evaluation and Treatment of Patients with Hypercortisolism: A Review. JAMA Surg 2020;155:1152-1159.
4. Lonser RR, Wind JJ, Nieman LK, Weil RJ, DeVroom HL, Oldfield EH. Outcome of surgical treatment of 200 children with cushing's disease. J Clin Endocrinol Metab 2013;98:892-901. Epub 2013 Jan 31
5. Moszczyńska E, Marczak E, Szalecki M, Kądziołka K, Roszkowski M, Zagata-Lesnicka P. The Effects of Sampling Lateralization on Bilateral Inferior Petrosal Sinus Sampling for Pediatric Cushing's Disease-A Single Endocrinology Centre Experience and Review of the Literature. Front Endocrinol (Lausanne) 2021;12:650967.
6. Virú-Loza MA, Diaz-Arocutipa C, Venegas A. Diagnostic accuracy of bilateral inferior petrosal sinus sampling with desmopressin for pediatric Cushing's disease: a systematic review and meta-analysis. PROSPERO, 2021. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID = CRD42021238718
7. Cavalcante LBCP, Freitas TC, Musolino NRC, Cescato VAS, Silva GO, Fragoso MCBV, Puglia P Jr, Bronstein MD, Machado MC. High accuracy of bilateral and simultaneous petrosal sinus sampling with desmopressin for the differential diagnosis of pediatric ACTH-dependent Cushing's syndrome. Pituitary 2020;23:507-514.
8. Chen S, Chen K, Lu L, Zhang X, Tong A, Pan H, Zhu H, Lu Z. The effects of sampling lateralization on bilateral inferior petrosal sinus sampling and desmopressin stimulation test for pediatric Cushing's disease. Endocrine 2019;63:582-591. Epub 2018 Oct 11
9. Machado MC, de Sa SV, Domenice S, Fragoso MC, Puglia P Jr, Pereira MA, de Mendonça BB, Salgado LR. The role of desmopressin in bilateral and simultaneous inferior petrosal sinus sampling for differential diagnosis of ACTH-dependent Cushing's syndrome. Clin Endocrinol (Oxf) 2007;66:136-142.
10. Gil S, Di Palma I, Vaiani E, Viterbo G, Requejo F, Ramos JG, Lazzati JM, Lubieniecki F, Carlos R, Ciaccio M. Use of desmopressin for bilateral inferior petrosal sinus sampling (BIPSS) in pediatric patients with Cushing disease (CD). Horm Res Paediatr 2019;91:251.
11. Kang KM, Muralidharan K, Knowlton H, Hassan KIA, Yekula A, Misra M, Swearingen B, Jones PS. Utility of bilateral inferior petrosal sinus sampling for diagnosis and lateralization of Cushing's disease in the pediatric population: case series and review of the literature. J Endocrinol Invest 2022;45:617-627. Epub 2021 Oct 15
12. Deipolyi A, Bailin A, Hirsch JA, Walker TG, Oklu R. Bilateral inferior petrosal sinus sampling: Experience in 327 patients. J Neurointerv Surg 2017;9:196-199. Epub 2016 Feb 15
13. Feng M, Liu Z, Liu X, Zhang X, Bao X, Yao Y, Deng K, Xing B, Lian W, Zhu H, Lu L, Wang R. Tumour lateralization in Cushing's disease by inferior petrosal sinus sampling with desmopressin. Clin Endocrinol (Oxf) 2018;88:251-257. Epub 2017 Nov 27

14. Pereira CA, Ferreira L, Amaral C, Alves V, Xavier J, Ribeiro I, Cardoso H. Diagnostic accuracy of Bilateral Inferior Petrosal Sinus Sampling: The Experience of a Tertiary Centre. Exp Clin Endocrinol Diabetes 2021;129:126-130. Epub 2019 Aug 19
15. Wind JJ, Lonser RR, Nieman LK, DeVroom HL, Chang R, Oldfield EH. The lateralization accuracy of inferior petrosal sinus sampling in 501 patients with Cushing's disease. J Clin Endocrinol Metab 2013;98:2285-2293. Epub 2013 Apr 3
16. Kanter AS, Diallo AO, Jane JA Jr, Sheehan JP, Asthagiri AR, Oskouian RJ, Okonkwo DO, Sansur CA, Vance ML, Rogol AD, Laws ER Jr. Single-center experience with pediatric Cushing's disease. J Neurosurg 2005;103(5 Suppl):413-420.
17. Joshi SM, Hewitt RJ, Storr HL, Rezajooi K, Ellamushi H, Grossman AB, Savage MO, Afshar F. Cushing's disease in children and adolescents: 20 years of experience in a single neurosurgical center. Neurosurgery 2005;57:281-285; discussion 281-285.

CASE REPORT

DOI: 10.4274/jcrpe.galenos.2020.2020.0100

J Clin Res Pediatr Endocrinol 2022;14(3):339-343

# Clinical Features in Patients with Xq23 Microdeletion: A Case Report and Literature Review

**Lu Qin[1], Fei-Zhou Zhang[1], Jian-Hai Lv[2], Lan-Fang Tang[2]**

*[1]Children's Hospital of Zhejiang University School of Medicine, Department of Pulmonology, Zhejiang, China*
*[2]Shangyu People's Hospital, Clinic of Pediatrics, Zhejiang, China*

**What is already known on this topic?**

Deletions in Xq22.3-q23 region may result in Alport syndrome, with intellectual disability, midface hypoplasia and elliptocytosis (*AMME*, MIM 300194), growth retardation, language delay, and alterations of bones, heart and eyes. Disorders affecting *AMMECR1* can cause midface hypoplasia, elliptocytosis, language disorder including early speech or language delay, infantile hypotonia, hearing loss, nephrocalcinosis and submucous cleft palate, as well as short stature, cardiac and skeletal abnormalities. Another gene, *CHRDL1*, has been identified as the causative gene of X-linked megalocornea (MGC1; MIM 309300), which is associated with distinctive secondary changes in the posterior crocodile shagreen and corneal arcus juvenilis.

**What this study adds?**

We report a boy with Xq23 microdeletion, which involved both the *AMMECR1* and *CHRDL1* genes who presented with microsomia, midface hypoplasia, kidney dysplasia, growth retardation and some alterations of bones and heart. Furthermore, patients with Xq23 microdeletion or a deletion overlapping these genes partially, was reviewed to highlight the rare condition and analyze the genotype-phenotype correlations.

## Abstract

Xq22.3-q23 microdeletion is a rare genomic disorder. The purpose of this study was to emphasize the correlation between clinical phenotype and genotype of proximal deletion on chromosome Xq22.3-q23. A 5 years old boy had a 671 KB microdeletion on Xq23 by chromosomal microarray analysis, including *AMMECR1* and *CHRDL1* genes. He presented with microsomia, midface hypoplasia, right kidney dysplasia and mildly motor retardation, which have not previously been reported in relation to Xq23 deletion. To the best of our knowledge, this is the first case with Xq23 microdeletion. A total of nine cases with microdeletion at Xq22.3-q23 affecting *AMMECR1* and two cases with *CHRDL1* mutation were reviewed. This review showed that Xq23 microdeletion with microsomia, midface hypoplasia, kidney dysplasia, and mild motor retardation was rare. The previous literature showed two novel point mutations in *AMMECR1* and *CHRDL1* with some phenotype difference from the presented case. Xq23 microdeletion should be considered for patients with microsomia, midface hypoplasia, kidney dysplasia and growth retardation.

**Keywords:** Xq23 microdeletion, midface hypoplasia, kidney dysplasia, growth retardation

## Introduction

Xq22.3-q23 microdeletion is a rare genomic disorder, which encompasses the entire *COL4A5* gene and its adjacent genes extending towards the telomere, including *GUCY2F*, *NXT2*, *KCNE1L*, *ACSL4*, *TMEM164*, *MIR3978*, *AMMECR1*, *SNORD96B*, *RGAG1*, *TDGF3*, *CHRDL1*, *PAK3* and *DCX* (1). Deletions in this region may present with Alport syndrome, with intellectual disability, midface hypoplasia and elliptocytosis (AMME, MIM 300194), growth retardation, language delay, and alterations of bones, heart and eyes. The differences in phenotype are probably associated with the location and size of the deletion, which may include different genes. With array CGH technology, more patients with Xq microdeletion have been reported. To date, about 11 families and 21 patients with microdeletion at Xq22.3-q23

**Address for Correspondence:** Lan-Fang Tang MD, Shangyu People's Hospital, Clinic of Pediatrics, Zhejiang, China
**Phone:** +86-13868138022 **E-mail:** 6195007@zju.edu.cn **ORCID:** orcid.org/0000-0002-7994-7066

**Conflict of interest:** None declared
**Received:** 27.05.2020
**Accepted:** 07.12.2020

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

J Clin Res Pediatr Endocrinol
2022;14(3):339-343

have been reported. However, only two have been reported when the microdeletion has affected the *AMMECR1* (MIM 300990) and *Chordin-like 1* (*CHRDL1* MIM 300350) genes, respectively. *AMMECR1* maps within the AMME complex interval (2). Disorder of this gene can cause midface hypoplasia, elliptocytosis, language disorder including early speech or language delay, infantile hypotonia, hearing loss, nephrocalcinosis and submucous cleft palate. In addition, *AMMECR1* is also associated with short stature, cardiac and skeletal abnormalities (2). *CHRDL1* has been identified as the causative gene of X-linked megalocornea (MGC1; MIM 309300), which is associated with distinctive secondary changes in the posterior crocodile shagreen and corneal arcus juvenilis (3).

Herein, we report a patient with Xq23 microdeletion, which involved both the *AMMECR1* and *CHRDL1* genes who presented with microsomia, midface hypoplasia, kidney dysplasia, growth retardation and some alterations of bones and heart. Furthermore, patients with Xq23 microdeletion or a deletion overlapping these genes partially, was reviewed to highlight the rare condition and analyze the genotype-phenotype correlations.

## Case Report

A 5-year-old boy presented to our clinic for developmental delay with mildly motor retardation for 5 years. He weighed 3.3 kg at birth without any suspicion of asphyxia. At presentation he was Tanner stage $G_2P_2$ of nonconsanguineous, healthy parents. Antenatal screening implied right renal dysplasia and regular postpartum re-examination confirmed the diagnosis. Growth retardation with short stature were noted after birth. He was able to sit at the age of 9 months and walk at the age of 19 months. He could use simple words, such as "mum", at about 11 months but still stuttered. His right kidney was surgically removed at the aged of 3 years 8 months. During the operation, thin right ureter and multiple cysts in the left kidney were noted. Electrocardiogram and cardiac ultrasound examination showed type B pre-excitation syndrome and acleistocardia (Φ 0.27 cm). Chest X-ray revealed no obvious abnormality of cardiopulmonary structures or diaphragm. Abdominal ultrasound scan did not show any abnormality of the liver, gallbladder or spleen. His father and mother were 163 cm and 153 cm, respectively while his 8 year-old sister was also growing slowly [about 123 cm, <-1 standard deviation (SD)]. There was no family history of hereditary disease in the patient's family.

On physical examination, his height was 99.3 cm (<-2 SD). In addition, he had characteristic features, comprising flat facial profile, mildly epicanthic folds, downslanting palpebral fissures, flat nasal bridge, bulbous nose, smaller left cheek, and slightly asymmetrical nasolabial sulcus (Figure 1A). A flat lateral top of the skull, occipital protuberance, low-set ears, low hairline and short neck were also noted (Figure 1B). In addition, malpositioned teeth and bilateral clinodactyly of the fifth finger were also noted (Figure 1C). However, his intelligence was normal.

Laboratory examination of thyroid, liver and kidney function, gas chromatography/mass spectrometry for blood were regular or negative. X-ray scan showed seven ossification centers in the right-hand carpal bones. Chromosomal microarray analysis found a 671 KB microdeletion located at Xq23, which involved both the *AMMECR1* and *CHRDL1* genes (Figure 2). Unfortunately, the parents refused to perform further genetic testing.

We reviewed the literature in English (PubMed and OMIM database) and Chinese. A total of nine cases with

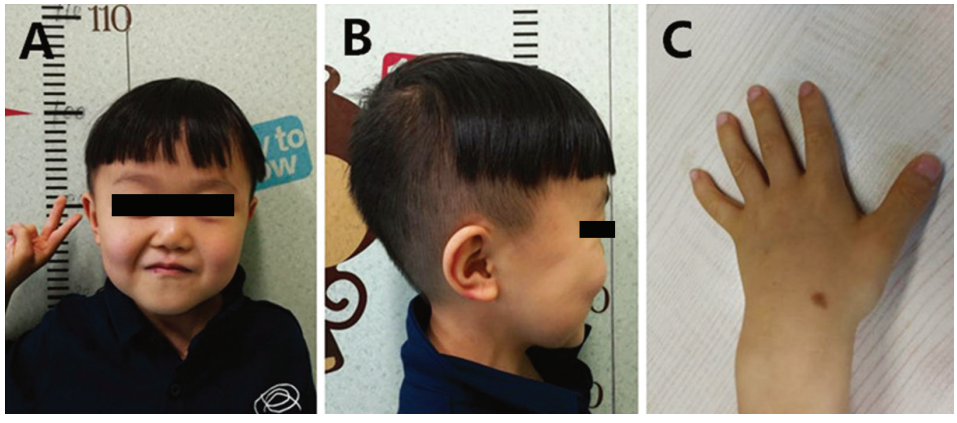


**Figure 1.** Clinical photographs of the current case. (A) Flat facial profile, mildly epicanthic folds, downslanting palpebral fissures, flat nasal bridge, bulbous nose, and short neck. The left cheek is smaller than the right cheek, and there is a slightly asymmetry of the nasolabial sulcus. (B) Flat lateral top of the skull, occipital protuberance, low-set ears and low hairline. (C) Clinodactyly of the fifth finger

microdeletion at Xq22.3-q23 involving the *AMMECR1* gene and two cases with *CHRDL1* mutation were founded. The clinical features of all reported cases are shown in Table 1.

## Discussion

We found a 671 KB microdeletion located at Xq23, which involved both the *AMMECR1* and *CHRDL1* genes. Although Xq22.3-q23 microdeletion has been reported, to our knowledge, this is the first case only with Xq23 microdeletion. Literature describing disorders associated with *AMMECR1* is also rare. The description and name of the gene (Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1) is derived from its cytogenic location within Xq22.3-q23 and its previous association with a very peculiar contiguous gene deletion syndrome, originally named AMME (Alport syndrome, mental retardation, midface hypoplasia and eliptocytosis). A deletion including *COL4A5* (Xq22.3) extending proximally to include *AMMECR1* which was first named AMME (4). However, the biological function of *AMMECR1* remains unclear. It was reported to code a protein that has a putative nuclear localization signal and may, therefore, encode a factor that regulates transcription (5). Besides, a 250 KB deletion on Xq23 that involved the *CHRDL1* gene and segregated with the affected phenotype in the affected individual's family, these authors described an inherited congenital disorder which was named MGC1. This gene encodes ventroptin, a bone morphogenic protein antagonist, with a proposed role in specification of topographic retinotectal projections and *CHRDL1* is differentially expressed in the human fetal brain, with high expression in cerebellum and neocortex (6).

Similar to two previously reported cases with the same mutation at Xq22.3-q23 of the *AMMECR1* gene by Andreoletti et al. (1) the current case presented with microsomia, right kidney dysplasia, mild motor retardation, spoke early but with a stutter, had midface hypoplasia including flat facial profile, mildly epicanthic folds, downslanting palpebral fissures, flat nasal bridge, bulbous nose, and a short neck. This implies that in patients with growth retardation, midface hypoplasia, kidney dysplasia, motor retardation, and language disorder, as well as alteration of bones and heart, Xq23 microdeletion should be considered.

Comparing the phenotype of the current patient with previous cases, we noted that the current case did not show mental retardation or hearing loss and showed a language disorder, which was noted in patients reported by Jonsson et al. (4). However, the current case also presented with bilateral clinodactyly of the fifth finger, cardiac and skeletal abnormalities but did not have elliptocytosis or hearing loss, which was noted in the two cases reported by Andreoletti et al. (1). These authors detailed varying degrees of hearing impairment but without skeletal abnormalities. However, the older brother of these two cases showed scattered elliptocytes and anisocytosis. It was remarkable that the current case presented with short stature, skeletal and cardiac abnormalities, similar to the five cases reported by Moysés-Oliveira et al. (2) but the previous reported patients also had hearing loss while the current case did not. This


**Figure 2.** Chromosomal microarray analysis found one 671 KB microdeletion located at Xq23 that covered the *AMMECR1* and *CHRDL1* genes

**Table 1. Clinical findings in patients with Xq22.3-23 deletion including *AMMECR1* or partially overlapping this region**

| Case & Reference | Case 1 (2) | Case 2 (2) | Case 3 (1) | Case 4 (1) | Case 5 (4) | Case 6 (4) | Case 7 (4) | Case 8 (4) | Case 9 (4) | Current case |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex | Male | Male | Male | Male | Female | Male | Male | Male | Male | Male |
| Gene | *COL4A6* to *TDGF3* | *COL4A6* to *TDGF3* | *AMMECR1* | *AMMECR1* | *AMMECR1* | *AMMECR1* | *AMMECR1* | *AMMECR1* | *AMMECR1* | *AMMECR1* to *CHRDL1* |
| Short stature | + | + | + | + | + | + | + | + | – | + |
| Hypotonia | + | + | + | + | NI | + | + | NI | NI | – |
| Hearing loss | + | + | + | + | + | + | + | NI | NI | – |
| Mental retardation | – | – | NI | NI | – | + | – | + | + | – |
| Motor delay | + | + | + | + | – | + | – | – | + | + |
| Midface hypoplasia | + | + | + | + | + | + | + | – | + | + |
| Ocular abnormality | + | + | – | + | NI | NI | NI | NI | NI | – |
| Language disorder | delay | delay | Early & delay | + | NI | NI | NI | NI | delay | stuttered |
| Kidney abnormality | haematuria | haematuria | nephrocalcinosis | nephrocalcinosis | – | + | – | – | – | dysplasia |
| Cardiac & skeletal abnormality | + | + | – | – | + | + | + | + | – | + |
| Elliptocytosis | + | + | – | – | – | – | – | – | + | – |

+: present, -: absent, NI: not investigated

finding on our patient suggested the microdeletion of Xq23 including *AMMECR1* may not be directly related to mental retardation and hearing loss, but it has a direct impact on growth and development, leading to facial malformation and bone and heart alterations. Besides, the phenotype of kidney abnormalities may be related to *AMMECR1*, but the manifestations of renal pathology are different. Moreover, the current case did not present with megalocornea or other eye lesions that have been associated with mutation of *CHRDL1* gene (7). It was notable that the report of Davidson et al. (8) included global developmental delay, midface hypoplasia, walking at the age of 20 months and a need for speech therapy in patients with mutation of *CHRDL1*. Our patient did not have intellectual disability at assessment while that of Davidson et al. (8) had moderate intellectual disability at the age of 10 years. Of course, the current patient may develop mental retardation later. Thus, given the clinical findings in the current case, microdeletion of Xq23 involving *CHRDL1* does not necessarily lead to X-linked megalocornea and intellectual disability. However, mutation of *CHRDL1* may lead to the above phenotypes. Besides, comparing the reports of Webb et al. (6) and Davidson et al. (8) with the current patient, we can hypothesize that megalocornea-mental retardation (MMR syndrome), in some cases, may be di- or multi-genic, because the current patient did not show any ocular abnormality and mental retardation. The clinical features of Xq22 to Xq23 microdeletion appear to be varied, although development disability, facial malformation, renal abnormality, cardiac and bone alteration are prevalent among individuals with deletions across the Xq22.3-q23 region.

## Conclusion

In summary, Xq23 microdeletion is a rare condition. In patients with growth retardation, midface hypoplasia, kidney dysplasia, motor retardation, and language disorder, as well as alteration of bones and cardiac morphology/function, Xq23 microdeletion should be considered. A detailed family history, careful physical exam to identify distinctive clinical features and improved genetic diagnosis may directly benefit the patient by allowing management and counseling specific for the disorder (9).

### Acknowledgement

We thank the patient and his parent to allow us using the data.

### Ethics

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

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Lu Qin, Concept: Fei-Zhou Zhang, Design: Lu Qin, Data Collection or Processing: Jian-Hai Lv, Analysis or Interpretation: Fei-Zhou Zhang, Literature Search: Lu Qin, Lan-Fang Tang, Writing: Lu Qin.

**Financial Disclosure:** This work is supported by the National Natural Science Foundation of China (81170016 & 81470214) and Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (2016).

## References

1. Andreoletti G, Seaby EG, Dewing JM, O'Kelly I, Lachlan K, Gilbert RD, Ennis S. AMMECR1: a single point mutation causes developmental delay, midface hypoplasia and elliptocytosis. J Med Genet 2017;54:269-277. Epub 2016 Nov 3
2. Moysés-Oliveira M, Giannuzzi G, Fish RJ, Rosenfeld JA, Petit F, Soares MF, Kulikowski LD, Di-Battista A, Zamariolli M, Xia F, Liehr T, Kosyakova N, Carvalheira G, Parker M, Seaby EG, Ennis S, Gilbert RD, Hagelstrom RT, Cremona ML, Li WL, Malhotra A, Chandrasekhar A, Perry DL, Taft RJ, McCarrier J, Basel DG, Andrieux J, Stumpp T, Antunes F, Pereira GJ, Neerman-Arbez M, Meloni VA, Drummond-Borg M, Melaragno MI, Reymond A. Inactivation of AMMECR1 is associated with growth, bone, and heart alterations. Hum Mutat 2018;39:281-291. Epub 2017 Dec 14
3. Mangialavori D, Colao E, Carnevali A, Bruzzichessi D, Grillone T, Perrotti N, Iuliano R, Scorcia V. Novel Mutation in the CHRDL1 Gene Detected in Patients With Megalocornea. Cornea 2015;34:976-979.
4. Jonsson JJ, Renieri A, Gallagher PG, Kashtan CE, Cherniske EM, Bruttini M, Piccini M, Vitelli F, Ballabio A, Pober BR. Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis: a new X linked contiguous gene deletion syndrome? J Med Genet 1998;35:273-278.
5. Vitelli F, Piccini M, Caroli F, Franco B, Malandrini A, Pober B, Jonsson J, Sorrentino V, Renieri A. Identification and characterization of a highly conserved protein absent in the Alport syndrome (A), mental retardation (M), midface hypoplasia (M), and elliptocytosis (E) contiguous gene deletion syndrome (AMME). Genomics 1999;55:335-340.
6. Webb TR, Matarin M, Gardner JC, Kelberman D, Hassan H, Ang W, Michaelides M, Ruddle JB, Pennell CE, Yazar S, Khor CC, Aung T, Yogarajah M, Robson AG, Holder GE, Cheetham ME, Traboulsi EI, Moore AT, Sowden JC, Sisodiya SM, Mackey DA, Tuft SJ, Hardcastle AJ. X-linked megalocornea caused by mutations in CHRDL1 identifies an essential role for ventroptin in anterior segment development. Am J Hum Genet 2012;90:247-259. Epub 2012 Jan 26
7. Han J, Young JW, Frausto RF, Isenberg SJ, Aldave AJ. X-linked Megalocornea Associated with the Novel CHRDL1 Gene Mutation p.(Pro56Leu*8). Ophthalmic Genet 2015;36:145-148. Epub 2013 Sep 27
8. Davidson AE, Cheong SS, Hysi PG, Venturini C, Plagnol V, Ruddle JB, Ali H, Carnt N, Gardner JC, Hassan H, Gade E, Kearns L, Jelsig AM, Restori M, Webb TR, Laws D, Cosgrove M, Hertz JM, Russell-Eggitt I, Pilz DT, Hammond CJ, Tuft SJ, Hardcastle AJ. Association of CHRDL1 mutations and variants with X-linked megalocornea, Neuhäuser syndrome and central corneal thickness. PLoS One 2014;9:e104163.
9. Jee YH, Baron J, Nilsson O. New developments in the genetic diagnosis of short stature. Curr Opin Pediatr 2018;30:541-547.

CASE REPORT

DOI: 10.4274/jcrpe.galenos.2021.2020.0287

J Clin Res Pediatr Endocrinol 2022;14(3):344-349

# Left Ventricular Hypertrophy in Patients with X-Linked Hypophosphataemia

**Ana Castellano-Martinez[1], Silvia Acuñas-Soto[1], Virginia Roldan-Cano[1], Moises Rodriguez-Gonzalez[2]**

*[1]Puerta del Mar University Hospital, Department of Pediatric Nephrology, Cadiz, Spain*
*[2]Puerta del Mar University Hospital, Department of Pediatric Cardiology, Cadiz, Spain*

**What is already known on this topic?**

X-linked hypophosphataemia is a rare disorder with well-known mechanisms leading to low phosphorus levels, but it does not clearly explain the cardiovascular involvement of these patients. Burosumab is a novel therapy for this entity, but it is not yet known whether early treatment can prevent complications, including cardiac manifestations.

**What this study adds?**

We report two patients with X-linked hypophosphataemia under burosumab therapy, one of them with a more severe phenotype associated with cardiac damage in the form of left ventricular hypertrophy. This patient had pre-existing cardiac involvement while receiving conventional treatment, which was not reversed after the start of burosumab therapy, although there was no worsening after start of burosumab.

## Abstract

X-linked hypophosphatemia (XLH) is a rare genetic disorder with X-linked dominant inheritance. Mutations in the *PHEX* gene increase fibroblast growth factor 23 (FGF23) concentrations, causing loss of phosphorus at the proximal tubule. Most pediatric patients debut in the first two years with short stature and bowed legs. Conventional treatment consists of oral supplements with phosphorus and calcitriol. Since 2018, burosumab has been approved as a novel therapeutic option for XLH, with promising results. The purpose of this study was to share our experience with two cases of XLH treated with burosumab. These patients presented with a broad phenotypical differences. One had the most severe radiological phenotype and developed left ventricular hypertrophy (LVH) and left ventricular dysfunction with preserved ejection fraction. Treatment with burosumab was well-tolerated and was followed by radiological stability and a striking improvement in both blood biochemistry and quality of life. The LVH was stable and left ventricular function normalized in the patient with cardiac involvement. In recent years many studies have been carried out to explain the role of FGF23 in cardiovascular damage, but the exact pathophysiological mechanisms are as yet unclear. The most intensively studied populations are patients with XLH or chronic kidney disease, as both are associated with high levels of FGF23. To date, cardiovascular involvement in XLH has been described in patients treated with conventional treatment, so it would be of interest to investigate if early use of burosumab at the time of diagnosis of XLH would prevent the occurrence of cardiovascular manifestations.

**Keywords:** X-linked hypophosphataemia, FGF23, arterial hypertension, cardiovascular risk, left ventricular hypertrophy, burosumab

## Introduction

X-linked hypophosphataemia (XLH) is a dominant genetic disorder caused by mutations in the *PHEX* gene (Xp22.1) and constitutes one of the leading causes of inherited hypophosphataemia with a prevalence of 4.8 per 100,000 (1). The key physiopathological mechanism seems to be an increased production of fibroblast growth factor 23 (FGF23) by osteocytes and osteoblasts due to a loss of function in the *PHEX* gene (2). FGF23 acts on the proximal renal tubule binding to the Klotho-FGF receptor complex, inhibiting phosphate reabsorption and calcitriol production, leading to chronic hypophosphataemia (3).

**Address for Correspondence:** Ana Castellano-Martinez MD, Puerta del Mar University Hospital, Department of Pediatric Nephrology, Cadiz, Spain
**Phone:** +34 956002700 **E-mail:** anacastellanomart@gmail.com **ORCID:** orcid.org/0000-0001-7563-1235

**Conflict of interest:** None declared
**Received:** 01.12.2020
**Accepted:** 21.02.2021

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

Most pediatric patients with XLH manifest their first clinical symptoms in the first two years of life, presenting with disproportionate shortness and bone deformities, especially of the lower limbs, and craniosynostosis. Other complications, such as enthesopathy, osteomalacia, dental abscesses or hearing loss, often appear throughout the lives of these patients (4). There is a broad phenotypic spectrum even among family members. Consequently, a genotype-phenotype correlation is not well described (5). The conventional treatment consists of oral administration of phosphorus and calcitriol but these supplementary therapies do not inhibit the action of the high levels of FGF23, and they do not stop the progression of the disease.

In 2018, burosumab, a human monoclonal IgG1 antibody that neutralizes FGF23 was approved for the treatment of XLH (6). Since then, burosumab has shown promising results with clinical improvement and quality of life for these patients (7). Our aim was to share our experience with this novel treatment in two pediatric patients with different phenotypic patterns of XLH, focusing on cardiovascular complications in this population.

## Case Report

The first case is a male patient diagnosed with XLH at 22 months of age during the investigation of his shortness and bowing of limbs (Figure 1). Hypophosphataemia with hyperphosphaturia (Table 1) was detected, and mutations in the *PHEX* gene were examined by next-generation sequencing (NGS) (8) with detection of a hemizygotic mutation. Therefore, he was started on conventional oral treatment. Poor biochemical and radiographic control persisted, despite good therapeutic compliance, requiring hemi-epiphysiodesis at 4 years old due to the significant deformity in the lower limbs. This led to walking disturbance and there was no improvement whatsoever. At the age of six, a first cardiovascular assessment with ambulatory blood pressure monitoring (ABPM), electrocardiogram (ECG), and echocardiography was carried out. The ABPM and ECG demonstrated no alterations, but the echocardiography revealed a concentric, non-obstructive, left ventricular hypertrophy (LVH) and associated subclinical left ventricular dysfunction parameters. Multiple criteria for definition of LVH in this patient were assessed (Table 1). The left ventricular ejection fraction was in the normal range (>50%), but this parameter only assesses the systolic function and it is usually altered only in patients

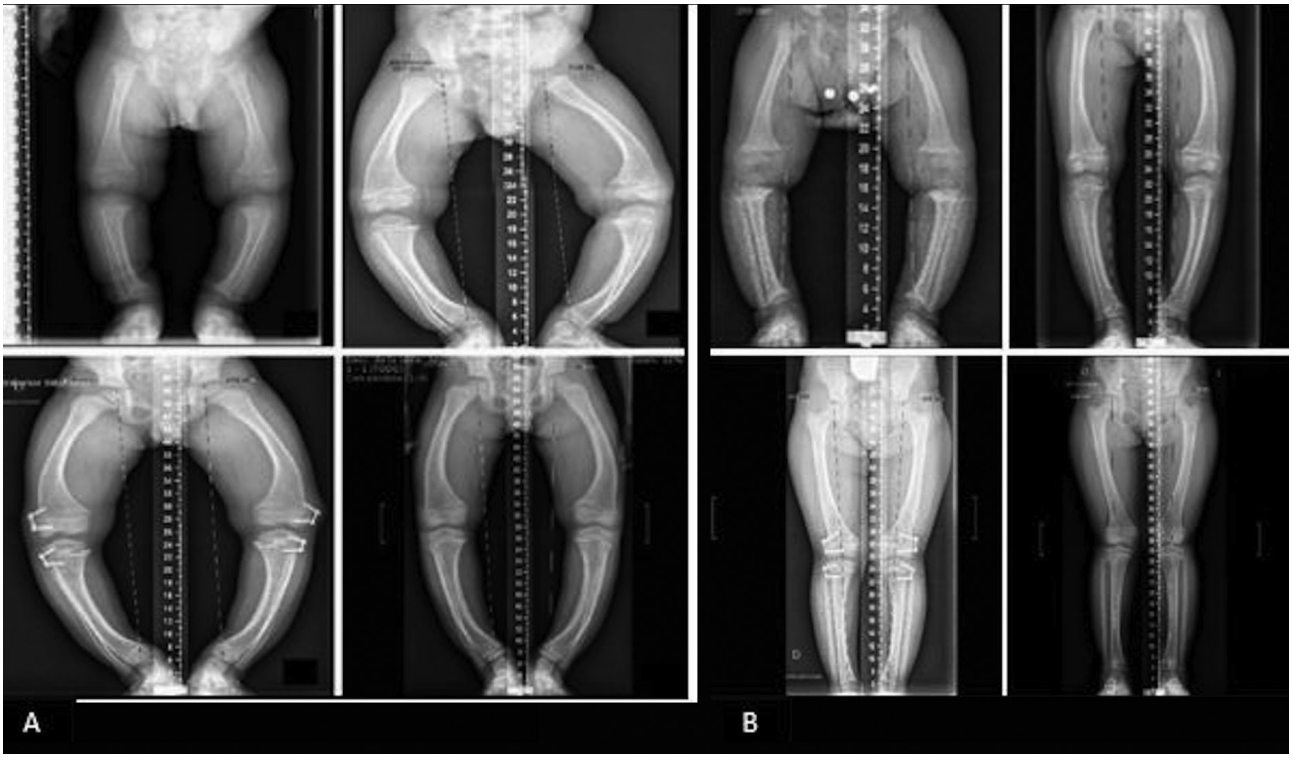


**Figure 1.** Radiological evolution of the patients. A) Evolution of patient 1, requiring hemiepiphysiodesis at 4 years-old for 19 months which was not effective, withdrawn after starting treatment with burosumab. Upper left panel (1.75 years); Upper right panel (4 years); Bottom left panel (6 years); Bottom right panel (8 years). B) Evolution of patient 2, with less lower limb deformity with a correction after hemiepiphysiodesis at 5.5 years-old during 9 months. Upper left panel (2 years); Upper right panel (5 years); Bottom left panel (6 years); Bottom right panel (9 years)

**Table 1. Evolution of clinical, biochemical and echocardiographic parameters of the patients at different stages of the disease**

| Variable | Patient 1 | | | Patient 2 | | |
|---|---|---|---|---|---|---|
| | Debut | Before burosumab | Last follow-up | Debut | Before burosumab | Last follow-up |
| **Age and anthropomorphic measures** | | | | | | |
| Age (yr) | 1.75 | 6 | 9 | 2 | 9 | 10 |
| Weight (kg), (SD) | 14.2 (+1.27) | 27.3 (+0.71) | 35 (+0.1) | 12 (-0.85) | 28.8 (-0.42) | 33 (-0.36) |
| Height (cm), (SD) | 83.5 (-2.32) | 111.7 (-1.83) | 129 (-1.27) | 79.7 (-2.99) | 116.5 (-2.9) | 124 (-2.42) |
| BSA ($m^2$, by Mosteller) | 0.57 | 0.92 | 1.12 | 0.51 | 0.96 | 1.06 |
| LBM (Kg, by Peters) | - | 21 | 27.4 | - | 22.5 | 25.7 |
| BMI ($kg/m^2$) | 20.4 | 21.9 | 21 | 18.9 | 21.2 | 21.5 |
| *PHEX* mutation | Hemizygosis c.670c>T(p.[Gln224*]) | | | Heterozygosis c.1061c>T(p.Pro534Leu) | | |
| **Laboratory (reference values)** | | | | | | |
| Calcium (mg/dL) | 9.2 (9.4-10.8) | 9.6 (9.4-10.3) | 9.2 (9.4-10.3) | 9.9 (9.4-10.8) | 10.2 (9.4-10.3) | 9.8 (9.3-10.3) |
| Phosphate (mg/dL) | 2.2 (4.5-6.5) | 2.5 (3.6-5.8) | 3.4 (3.6-2.8) | 2.4 (4.5-6.5) | 1.8 (3.6-5.8) | 3.5 (3.6-5.8) |
| ALP (40-462 mg/dL) | 539 | 576 | 313 | 486 | 397 | 307 |
| PTH (15-85 pg/mL) | 77 | 30.6 | 63.2 | 46.7 | 156.6 | 68 |
| 25-OH-vitamin D (21-100 ng/mL) | 24.9 | 29.4 | 40.7 | 21.3 | 24.9 | 39 |
| 1.25-OH-D-vitamin (16-56 pg/mL) | 106 | 44 | 42 | 30 | 36 | 41 |
| FGF23 c-terminal (<145 RU/mL) | >427 | >427 | >427 | >427 | >427 | >427 |
| TRP (>85%) | 70 | 54.4 | 86 | 80 | 30 | 98 |
| Tmp/GFR (mg/100 mL) | 1.556 (5.1±0.9) | 1.674 (4.6±0.6) | 3.311 (4.6±0.6) | 2.032 (5.1±0.9) | 0.976 (4.6±0.6) | 2.973 (4.6±0.6) |
| Ca/Cr (0.2 mg/mg) | 0.15 | 0.08 | 0.18 | 0.06 | 0.04 | 0.09 |
| Nephrocalcinosis | No | | | No | | |
| **Ambulatory blood pressure monitoring** | | | | | | |
| 24h mean SBP percentile | - | p86 | p89 | - | p90 | p89 |
| 24h mean DBP percentile | - | p70 | p77 | - | p89 | p84 |
| SBP load (%) | - | 12 | 15 | - | 13 | 10 |
| DBP load (%) | - | 14 | 11 | - | 9 | 11 |
| Nocturnal dipping | - | Yes | Yes | - | Yes | Yes |
| **Echocardiography** | | | | | | |
| Diameter of IVS for BSA, mm (Z-score) | - | 12 (3.44) | 11.5 (2.89) | - | 8 (1.46) | 8.5 (1.57) |
| LVM (g) | - | 115 | 126 | - | 60.3 | 72.5 |
| LVMI >51($g/m^2.7$) | | Yes | Yes | - | Yes | Yes |
| LVM (g)/BSA (LVH >115 in boys and >95 in girls) | | 125 | 112.5 | | 62.8 | 71 |
| LVM for BSA (Z-score) | - | 3.65 | 2.75 | - | -0.12 | 0.38 |
| $LVM/m^{2.7}$ (percentile for age) | - | 85.3 (>$99^{th}$) | 63.3 (>$99^{th}$) | - | 39.9 (95-$99^{th}$) | 40.5 (95-$99^{th}$) |
| $LVM/[(m^{2.16}) + 0.09]$ (>45 $g/m^{2.16}$=LVH) | - | 84.5 | 69.1 | - | 43.3 | 43.1 |
| LVM percentile for LBM | - | >$97^{th}$ | $93^{rd}$ | - | $5^{th}$ | $10^{th}$ |
| LV Tei index (>0.50=dysfunction) | - | 0.57 | 0.42 | - | 0.35 | 0.41 |
| LVEF (%) (<50%=dysfunction) | - | 63 | 67 | - | 65 | 67 |

For definition of LVH a total of five different criteria were assessed.

Yr: years, SD: standard deviation, BSA: body surface area, LBM: lean body mass, BMI: body mass index, ALP: alkaline phosphatase, PTH: parathyroid hormone, FGF23: fibroblast growth factor 23, TRP: tubular reabsorption of phosphate, Tmp/GFR: maximal tubular phosphate reabsorption per 100 mL of filtrate, Ca/Cr: urine calcium/creatinine ratio, ABPM: ambulatory blood pressure monitoring, SBP: systolic blood pressure, DBP: diastolic blood pressure, IVS: interventricular septum, LVMI: left ventricular mass index, LVM: left ventricle mass, LV: left ventricle, LVEF: left ventricular ejection fraction

with clinically evident heart failure. To evaluate subclinical ventricular dysfunction, the Tei index was used, a parameter that includes both systolic and diastolic time intervals to assess global cardiac dysfunction and detect early cardiac dysfunction in asymptomatic patients (9). The Tei index is an easily performable, recordable and reproducible parameter that is influenced by age, sex, heart rate, and cardiac load conditions. A Tei index $<0.5$ is the upper limit of normal (2 Z-scores), and our patient presented with a Tei index value of 0.57.

At this point in 2017, it was decided to start burosumab (0.7 mg/kg every 15 days) as compassionate treatment. After 3 years of treatment at a dose of 1.2 mg/kg every 15 days, the treatment has been well tolerated without significant side-effects. His serum phosphorus concentrations have increased to near normal values for his age. We have also noticed a slow progression of the lower limbs' deformities with a significant improvement in quality of life (Figure 1A). Regarding the cardiovascular involvement, the dimensions of the left ventricle have remained stable, with a persistent LVH but with relatively reduced left ventricular mass index (LVMI) (Table 1). The left ventricular function has normalized (Tei index $<0.5$).

The second case was a female patient diagnosed with XLH at 24 months old during a study of genu varus. She had hypophosphataemia with hyperphosphaturia (Table 1), and we found a heterozygous mutation in the *PHEX* gene by NGS. She received conventional treatment, with persisting hypophosphataemia and worsening of the lower limb deformities (Figure 1B). Thus, she required hemi-epiphysiodesis at 5.5 years-old, which was effective. The patient continued with hypophosphataemia and also very poor gastrointestinal tolerance to oral phosphorus. Therefore, we decided to start burosumab treatment (0.4 mg/kg every 15 days) at 9 years old (2019). ABPM, ECG and echocardiography were uneventful without signs of arterial hypertension, LVH, or left ventricular dysfunction. After 1.5 years, the treatment has been well tolerated. The hypophosphataemia has improved, she maintains good radiological progress and her intestinal symptoms have disappeared. The cardiovascular assessment remains normal. Informed consent was obtained from both patients' parents.

## Discussion

Herein, we present two patients with XLH with different phenotypic expression. According to his records, the patient with the most severe radiological phenotype was diagnosed with LVH and left ventricular dysfunction by tissue Doppler echocardiography.

The definition of LVH is still controversial in children with multiple and variable existing criteria. The most commonly employed methods are adjusted for body surface area (BSA) or height, usually to an allometric power as the relationship between left ventricular mass (LVM) to height is not linear. Daniels et al. (10) suggested the use of height$^{2.7}$ for indexing LVM based on studies relating LVM to lean body mass (LBM) in older children. This boy fulfilled the LVH definitions recommended by current pediatric arterial hypertension guidelines (11): LVMI $>51$ g/m$^{2.7}$, LVM $>115$ g/BSA and LVM $>95^{th}$ percentile for age (12). Some authors raise the question of whether a higher cut-off ($99^{th}$ vs $95^{th}$ percentile) should be used, and our patient also met this condition (13).

The utility of LVM/height$^{2.7}$ in children has been questioned due to the index variation in the lower age and lower height groups (14,15). Thus, XLH patients usually present a low height for their age, and these criteria could lead to an overestimation of LVMI and LVH diagnosis in this population. Chinali et al. (14) showed that the allometric power of 2.16 provides the best-fit model. This method provides normal reference values of LVMI, even for children under 140 cm of height, which is more appropriate for XLH patients. In particular, a LVMI greater than 45 g/m$^{2.16}$ would represent the $95^{th}$ percentile across the whole pediatric age range, independent of the sex and height, to identify LVH, and our patient also met this criterion. Other LVH criteria assessed in this case were a LVM $>2.5$ Z-scores for BSA (16) and an interventricular septum diastolic diameter $>2.5$ Z-scores for BSA (17).

The normalization of the LVM to various functions of height, BSA, or body mass index (BMI) can alter the interpretation and classification of LVH in children. LVM varies in proportion to LBM but is usually expressed relative to height or BSA, each of which functions as a surrogate for LBM. Foster et al. (15) provided normal percentiles of LVM for LBM, and our patient presented as $>97^{th}$ percentile of LVM for LBM in the first echocardiographic control. Despite the existing controversy and the absence of definitive LVH criteria, particularly in low height populations, we provided data that patient 1 met multiple criteria for LVH. Patient 2 only fulfilled the LVH criteria of LVMI $>51$ g/m$^{2.7}$ and LVMI (g/m$^{2.7}$) $>95^{th}$ percentile for age, reinforcing the need to use specific criteria for the correct assessment of LVH in this population.

XLH is actually associated with LVH and its relationship with FGF23 levels and arterial hypertension as causative mechanisms in this setting remain uncertain, as reflected by the controversial published literature. Takashi et al. (18) did not observe LVH in 10 adults with XLH. Pastor-Arroyo et al. (19) studied the increased risk of cardiovascular

disease in XLH mouse models (*PhexC733R*), which showed increased FGF23 and parathyroid hormone (PTH) levels, hypophosphataemia, low 1.25(OH)2 vitamin D levels, and low soluble Klotho, but not arterial hypertension, LVH or cardiac dysfunction. Similar results were found in the study of Liu et al. (20) in male Hyp mouse model of XLH. Hernández-Frías et al. (21) reported twenty-four pediatric patients with XLH and they observed LVH in 18%. They did not find correlation with FGF23 levels and only one case presented with associated arterial hypertension. Accordingly, although our patient with LVH showed the most severe radiological phenotype of XLH, both cases presented with high levels of FGF23 and normal ranges of blood pressure. Therefore, pathophysiological mechanisms different than increased FGF23 and arterial hypertension might be involved in the development of LVH in XLH patients.

However, recent evidence has suggested a role for FGF23 in the development of cardiovascular involvement in XLH, particularly LVH and hypertension. Studies in animal models showed that FGF23 increased intracellular calcium levels, stimulating cardiac muscle contractility, with subsequent LVH. Abnormal calcium deposition in the vascular tissue causing arterial hypertension and subsequent LVH in relation with increased FGF23 has also been reported (22,23). Nehgme et al. (24) studied thirteen patients with XLH with an average age of 13.5 years. They found LVH in 54% of cases, but they did not investigate the correlation with FGF23 levels. However, they proved that all patients with XLH had significantly higher diastolic blood pressure (DBP) than the control group at the peak of exercise ergometry. While the association of arterial hypertension with LVH was not assessed, these results suggest that XLH patients manifest specific vascular dysregulation in association with the development of LVH. It should be noted that in pediatric patients (1-18 years), hypertension is defined as average clinic-measured systolic blood pressure (SBP) or DBP ≥95$^{th}$ percentile (based on age, sex, and height percentiles). In recent years the American Academy of Paediatrics has updated the guidelines with new reference values for the pediatric population, which should be used to avoid misclassification of hypertensive patients (11,25). The relationship between elevated levels of FGF23 and cardiovascular impairment, including LVH, has been widely investigated outside the XLH setting. It is known that chronic kidney disease (CKD) is associated with high levels of FGF23 and a deficiency of the co-receptor Klotho. The cardiovascular affectation seems to be directly caused by FGF23 by activating the intermediate molecule FGFR4, as myocytes do not express Klotho (26). Mitsnefes et al. (27) investigated the association of increased FGF23 and LVH in 587 pediatric patients with mild-moderate CKD. Interestingly, they showed a significant relationship between these two variables independent of SBP values.

## Conclusion

Further studies would be necessary to clarify the exact mechanisms involved in developing cardiovascular manifestations in XLH. The LVH observed in one of our patients highlights the utility of serial cardiovascular assessment, including serial blood pressure determination, ECG and echocardiography, as well as the promotion of adequate control of cardiovascular risk factors in this population. In our short-term experience, burosumab is an excellent therapeutic option for XLH in children, as it improves their quality of life and allows rapid biochemical stabilization without significant side effects. As was documented, there is a hypothetical role for the use of burosumab in stabilizing the LVH and normalizing the left ventricular function in XLH patients. Burosumab did not have an effect on reversing already established LVH in the patient with this, but it could similarly slow the progression as it does with radiological manifestations. It would be of interest to investigate if early use of burosumab at the time of diagnosis of XLH would prevent the occurrence of cardiovascular manifestations.

### Ethics

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

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Ana Castellano-Martinez, Silvia Acuñas-Soto, Virginia Roldan-Cano, Moises Rodriguez-Gonzalez, Concept: Ana Castellano-Martinez, Moises Rodriguez-Gonzalez, Design: Ana Castellano-Martinez, Moises Rodriguez-Gonzalez, Data Collection or Processing: Silvia Acuñas-Soto, Virginia Roldan-Cano, Analysis or Interpretation: Silvia Acuñas-Soto, Virginia Roldan-Cano, Literature Search: Ana Castellano-Martinez, Silvia Acuñas-Soto, Virginia Roldan-Cano, Moises Rodriguez-Gonzalez, Writing: Ana Castellano-Martinez, Silvia Acuñas-Soto, Virginia Roldan-Cano, Moises Rodriguez-Gonzalez.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Haffner D, Emma F, Eastwood DM, Duplan MB, Bacchetta J, Schnabel D, Wicart P, Bockenhauer D, Santos F, Levtchenko E, Harvengt P, Kirchhoff M, Di Rocco F, Chaussain C, Brandi ML, Savendahl L, Briot K, Kamenicky P, Rejnmark L, Linglart A. Clinical practice

recommendations for the diagnosis and management of X-linked hypophosphataemia. Nat Rev Nephrol 2019;15:435-455.

2. Fukumoto S. FGF23-related hypophosphatemic rickets/osteomalacia: diagnosis and new treatment. J Mol Endocrinol 2021;66:R57-R65.
3. Giralt M, Chocron S, Ferrer R, Ariceta G. Plasma intact fibroblast growth factor 23 level is a useful tool for diagnostic approach of renal hypophosphatemia. Pediatr Nephrol 2021;36:1025-1028. Epub 2021 Jan 25.
4. Dahir K, Roberts MS, Krolczyk S, Simmons JH. X-Linked Hypophosphatemia: A New Era in Management. J Endocr Soc 2020;4:bvaa151.
5. Scialla JJ, Wolf M. Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease. Nat Rev Nephrol 2014;10:268-278. Epub 2014 Apr 1
6. Padidela R, Cheung MS, Saraff V, Dharmaraj P. Clinical guidelines for burosumab in the treatment of XLH in children and adolescents: British paediatric and adolescent bone group recommendations. Endocr Connect 2020;9:1051-1056.
7. Martín Ramos S, Gil-Calvo M, Roldán V, Castellano Martínez A, Santos F. Positive Response to One-Year Treatment With Burosumab in Pediatric Patients With X-Linked Hypophosphatemia. Front Pediatr 2020;8:48.
8. Thiele S, Werner R, Stubbe A, Hiort O, Hoeppner W. Validation of a next-generation sequencing (NGS) panel to improve the diagnosis of X-linked hypophosphataemia (XLH) and other genetic disorders of renal phosphate wasting. Eur J Endocrinol 2020;183:497-504.
9. Cui W, Roberson DA. Left ventricular Tei index in children: comparison of tissue Doppler imaging, pulsed wave Doppler, and M-mode echocardiography normal values. J Am Soc Echocardiogr 2006;19:1438-1445.
10. Daniels SR, Kimball TR, Morrison JA, Khoury P, Witt S, Meyer RA. Effect of lean body mass, fat mass, blood pressure, and sexual maturation on left ventricular mass in children and adolescents. Statistical, biological, and clinical significance. Circulation 1995;92:3249-3254.
11. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, de Ferranti SD, Dionne JM, Falkner B, Flinn SK, Gidding SS, Goodwin C, Leu MG, Powers ME, Rea C, Samuels J, Simasek M, Thaker VV, Urbina EM; SUBCOMMITTEE ON SCREENING AND MANAGEMENT OF HIGH BLOOD PRESSURE IN CHILDREN. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. Pediatrics 2017;140:e20171904. Epub 2017 Aug 21
12. Khoury PR, Mitsnefes M, Daniels SR, Kimball TR. Age-specific reference intervals for indexed left ventricular mass in children. J Am Soc Echocardiogr 2009;22:709-714. Epub 2009 May 7
13. Gidding SS. Assessment of Left Ventricular Mass in Children and Adolescents: Current Status. J Pediatr 2016;170:12-14. Epub 2015 Dec 31
14. Chinali M, Emma F, Esposito C, Rinelli G, Franceschini A, Doyon A, Raimondi F, Pongiglione G, Schaefer F, Matteucci MC. Left Ventricular Mass Indexing in Infants, Children, and Adolescents: A Simplified Approach for the Identification of Left Ventricular Hypertrophy in Clinical Practice. J Pediatr 2016;170:193-198. Epub 2015 Dec 6
15. Foster BJ, Khoury PR, Kimball TR, Mackie AS, Mitsnefes M. New Reference Centiles for Left Ventricular Mass Relative to Lean Body Mass in Children. J Am Soc Echocardiogr 2016;29:441-447. Epub 2016 Feb 3
16. Foster BJ, Mackie AS, Mitsnefes M, Ali H, Mamber S, Colan SD. A novel method of expressing left ventricular mass relative to body size in children. Circulation 2008;117:2769-2775. Epub 2008 May 19
17. Pettersen MD, Du W, Skeens ME, Humes RA. Regression equations for calculation of z scores of cardiac structures in a large cohort of healthy infants, children, and adolescents: an echocardiographic study. J Am Soc Echocardiogr 2008;21:922-934. Epub 2008 Apr 11
18. Takashi Y, Kinoshita Y, Hori M, Ito N, Taguchi M, Fukumoto S. Patients with FGF23-related hypophosphatemic rickets/osteomalacia do not present with left ventricular hypertrophy. Endocr Res 2017;42:132-137. Epub 2016 Oct 18
19. Pastor-Arroyo EM, Gehring N, Krudewig C, Costantino S, Bettoni C, Knöpfel T, Sabrautzki S, Lorenz-Depiereux B, Pastor J, Strom TM, Hrabě de Angelis M, Camici GG, Paneni F, Wagner CA, Rubio-Aliaga I. The elevation of circulating fibroblast growth factor 23 without kidney disease does not increase cardiovascular disease risk. Kidney Int 2018;94:49-59. Epub 2018 May 5
20. Liu ES, Thoonen R, Petit E, Yu B, Buys ES, Scherrer-Crosbie M, Demay MB. Increased Circulating FGF23 Does Not Lead to Cardiac Hypertrophy in the Male Hyp Mouse Model of XLH. Endocrinology 2018;159:2165-2172.
21. Hernández-Frías O, Gil-Peña H, Pérez-Roldán JM, González-Sanchez S, Ariceta G, Chocrón S, Loza R, de la Cerda Ojeda F, Madariaga L, Vergara I, Fernández-Fernández M, Ferrando-Monleón S, Antón-Gamero M, Fernández-Maseda Á, Luis-Yanes MI, Santos F. Risk of cardiovascular involvement in pediatric patients with X-linked hypophosphatemia. Pediatr Nephrol 2019;34:1077-1086. Epub 2019 Jan 4
22. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-O M, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. J Clin Invest 2011;121:4393-4408. Epub 2011 Oct 10
23. Okamoto Y, Fujita S, Morita H, Kizawa S, Ito T, Sakane K, Sohmiya K, Hoshiga M, Ishizaka N. Association between circulating FGF23, α-Klotho, and left ventricular diastolic dysfunction among patients with preserved ejection fraction. Heart Vessels 2016;31:66-73. Epub 2014 Sep 16
24. Nehgme R, Fahey JT, Smith C, Carpenter TO. Cardiovascular abnormalities in patients with X-linked hypophosphatemia. J Clin Endocrinol Metab 1997;82:2450-2454.
25. Macumber I. Ambulatory Blood Pressure Monitoring in Children and Adolescents: a Review of Recent Literature and New Guidelines. Curr Hypertens Rep 2017;19:96.
26. Haffner D, Leifheit-Nestler M. Extrarenal effects of FGF23. Pediatr Nephrol 2017;32:753-765. Epub 2016 Oct 4
27. Mitsnefes MM, Betoko A, Schneider MF, Salusky IB, Wolf MS, Jüppner H, Warady BA, Furth SL, Portale AA. FGF23 and Left Ventricular Hypertrophy in Children with CKD. Clin J Am Soc Nephrol 2018;13:45-52. Epub 2017 Oct 12

CASE REPORT

DOI: 10.4274/jcrpe.galenos.2021.2020.0301

J Clin Res Pediatr Endocrinol 2022;14(3):350-355

# Differential Diagnosis of Acromegaly: Pachydermoperiostosis Two New Cases from Turkey

**Emine Kartal Baykan[1], Ayberk Türkyılmaz[2]**

*[1]Erzurum Regional Training and Research Hospital, Clinic of Endocrinology and Metabolic Diseases, Erzurum, Turkey*
*[2]Karadeniz Technical University Faculty of Medicine, Department of Medical Genetics, Trabzon, Turkey*

**What is already known on this topic?**

Pachydermoperiostosis (PDP) is a rare condition characterised by digital clubbing, joint problems and pachydermia, but other skin manifestations due to dermal and sebaceous gland hypertrophy can be found.

**What this study adds?**

PDP cases mimicking acromegaly are reported to bring attention to this differential diagnosis. Although both acromegaly and PDP are infrequently encountered, avoidance of diagnostic confusion is important because of the prognostic and therapeutic implications. Awareness of the significance of clubbing under these circumstances is likely to prevent misdiagnosis.

## Abstract

Pachydermoperiostosis (PDP), also known as primary hypertrophic osteoarthropathy, is a rare genetic disorder characterized by pachyderma and periostosis. Acromegaly is a condition caused by excessive secretion of growth hormone (GH) leading to elevated insulin-like growth factor 1 levels, and is characterised by somatic overgrowth and physical disfigurement, notably affecting hands and feet. We present two cases referred with an initial diagnosis of acromegaly that were ultimately diagnosed as PDP. Case 1: A 17 year-old boy presented with enlargement in both feet and hands, finger clubbing, swelling in knee joints, knee pain, coarsening of facial skin lines and forehead skin, and excessive sweating which increased gradually over five years. There were prominent skin folds on the forehead, face, and eyelids. Also, there was an enlargement in both hands and clubbing of the fingers. There was marked swelling in the knee joints and ankles. Genetic analysis revealed a novel homozygous variant NM_005630: c.31C>T (p.Q11*) in the *SLCO2A1* gene. Case 2: A 16 year-old boy presented with coarsening of forehead skin and scalp, excessive sweating, and pain in the elbow and knee over three years. Skin folds were prominent on the forehead and scalp. Genetic analysis revealed a homozygous variant NM_005630.2:c.86delG (p.G29Afs*48) in the *SLCO2A1* gene. Such clinical presentation contemporaneous with normal GH level and prominent radiological abnormalities prompted the diagnosis of PDP. In conclusion, PDP is a very rare osteoarthrodermopathic disorder with clinical and radiographic presentation that may mimic acromegaly. In the evaluation of patients with acromegaloid appearance, PDP should be considered as a differential diagnosis.

**Keywords:** Acromegaly, pachydermoperiostosis, diagnosis

## Introduction

Pachydermoperiostosis (PDP), also known as primary hypertrophic osteoarthropathy, is a rare genetic disorder characterized by pachyderma and periostosis. In 1935, the disorder was classified into three forms by three French dermatologists, Touraine, Solente, and Gole: complete (pachyderma and periostosis), incomplete (without pachyderma), and rough (pachyderma and minimal skeletal changes) forms (1). Diagnosis should be made when two of the following features are present: positive family history, hypertrophic skin changes, osseous pain/radiographic changes, or clubbing (2). Although the exact incidence is unknown in PDP, its prevalence is estimated at 0.16% (3). It generally manifests at puberty and the male:female ratio is 7:1 (4). The clinical features, signs and symptoms have

**Address for Correspondence:** Emine Kartal Baykan MD, Erzurum Regional Training and Research Hospital, Clinic of Endocrinology and Metabolic Diseases, Erzurum, Turkey
**E-mail:** emnkrtl@hotmail.com **ORCID:** orcid.org/0000-0001-6813-883X

**Conflict of interest:** None declared
**Received:** 09.12.2020
**Accepted:** 29.04.2021

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

close similarities with acromegaly and can cause diagnostic confusion (5). We present two cases referred with an initial diagnosis of acromegaly and were ultimately diagnosed as PDP.

## Case Reports

### Case 1

A 17 year-old boy initially presented to the family doctor with enlargement in both feet and hands and excessive sweating over five years, and he was referred to an endocrine outpatient clinic with an initial diagnosis of acromegaly. The patient's parents are cousins and the patient has a brother and a sister. There was no finding of similar complaints in their family history. The anamnesis revealed that his complaints had been worsening gradually over five years and that there was an enlargement in both feet and hands, clubbing in the fingers, swelling in knee joints, knee pain, coarsening of facial lines, particularly in the forehead skin, and excessive sweating. On physical examination, anthropometric measurements were: height, 183 cm [standard deviation score (SDS: 1.24)]; body weight, 79 kg (SDS: 0.89); and fathom distance, 183 cm. There were prominent skin folds on the forehead, face, and eyelids (Figure 1). There was also an enlargement in both hands and clubbing of the fingers (Figure 1). There was marked swelling in knee joints and ankles (Figure 1). Secondary sex characteristics were normal and the Tanner stage was five. Systemic examination, including cardiovascular and respiratory systems, neurological examination, and thyroid was normal. There was no scaling on the scalp, rash, psoriatic nail changes, subcutaneous nodules, or red-eye on physical examination. Complete blood count, hepatic and renal functions were within the normal range. Thyroid functions, follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, prolactin, rheumatoid factor, and anti-cyclic citrullinated peptide were normal. Blood gas analysis showed: pH, 7.36; $HCO_3$, 24.7; and $sO_2$, 98%. C-reactive protein was increased at 39.8 mg/L (reference: 0-5). Serological tests were performed to evaluate connective tissue disorders and vasculitis, which were all unremarkable. As acromegaly was suspected, insulin-like growth factor-1 (IGF-1) and growth hormone (GH) measurements were performed and the results were IGF-1 235 ng/mL (normal: 111-509) and fasting GH 0.35 mcg/L (normal: 0.07-5). The oral glucose tolerance test (OGTT) was normal, suppression of GH less than 1 µg/L. On bilateral hand radiographs, periostitis and hyperostosis were detected at the metacarpal and proximal phalanges

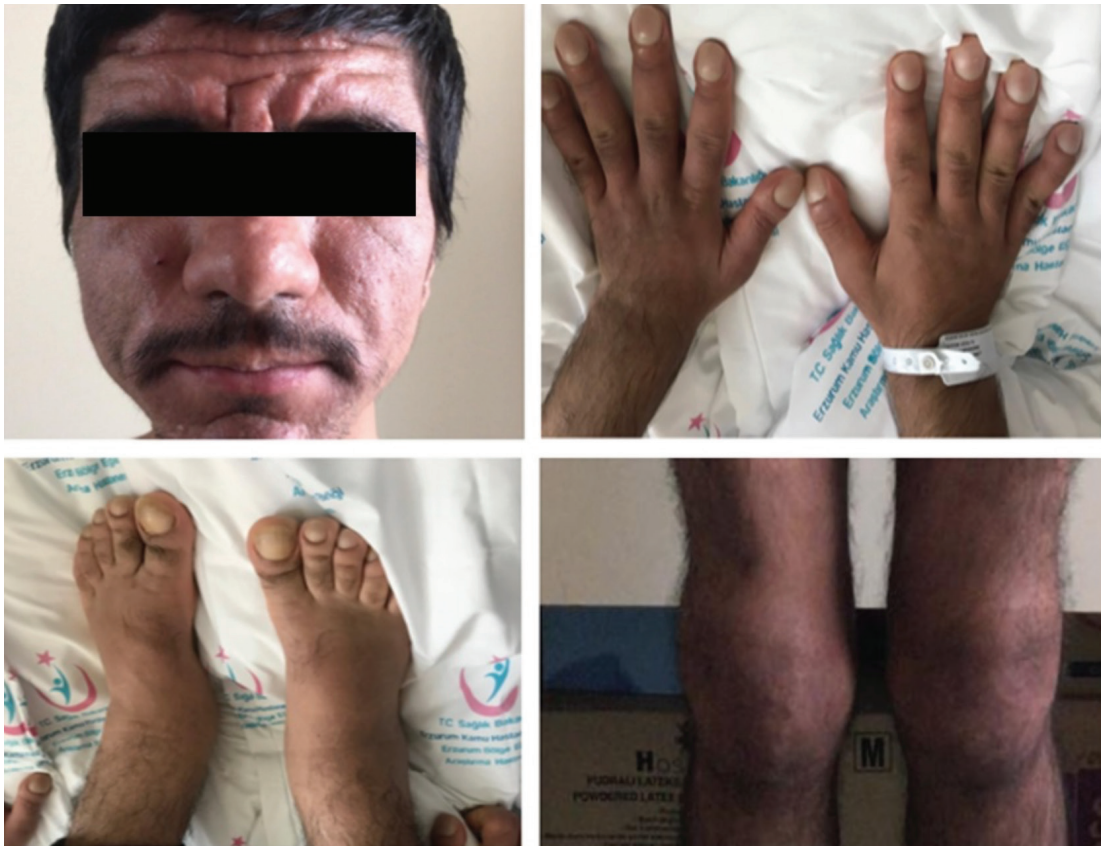

**Figure 1.** Physical examination findings of the first case

(Figure 2). *De novo* bone formation and cortical thickening were detected on bilateral knee radiographs (Figure 2). Irregular sub-periosteal *de novo* bone formation and cortical thickening were observed in the tibia, fibula, calcaneum, and talus on bilateral ankle radiographs (Figure 3). No pituitary adenoma was detected on pituitary magnetic resonance imaging (MRI). Chest radiograph and echocardiography were considered normal. No abnormal finding was detected in abdominal and thoracic computed tomography (CT) scans. The patient was diagnosed as a classical PDP, based on clinical, biochemical, and radiological findings. Genetic analysis revealed a novel homozygous nonsense variant NM_005630:c.31C > T (p.Q11 *) in exon 1 of the solute carrier organic anion transporter family member 2A1 (*SLCO2A1*) gene. Thus, selective Cox-2 inhibitor (oral meloxicam, 15 mg twice daily) and steroid (oral methylprednisolone, 5 mg/day) were prescribed. Marked improvement was detected in joint pain, swelling, and sweating in the first month after start of treatment. In the control visit at six months, regression was detected in the thickening at the forehead.

## Case 2

An 16 year-old boy with coarsening of forehead skin and scalp, excessive sweating, and pain in the elbow and knee over three years was referred to an endocrine outpatient clinic with an initial diagnosis of acromegaly. There was no finding of a similar disorder in the family history, nor consanguinity between parents. The patient has a sister. On physical examination, anthropometric measurements were: height, 176 cm (SDS: 0.39); body weight, 69 kg (SDS: 0.27); and fathom distance, 176 cm. Skin folds were prominent on the forehead skin and scalp (Figure 4). No abnormal finding was detected in the examination of the elbow and knee joint. Secondary sex characteristics were normal and the Tanner stage was five. Systemic examinations, including cardiovascular and respiratory systems, neurological examination, and thyroid, were normal. Complete blood count, hepatic and renal functions were within the normal range. Blood gas analysis showed: pH, 7.38; $HCO_3$, 23.8; and $sO_2$, 99%. C-reactive protein was found to be increased at 11.4 mg/L (reference: 0-5). Thyroid functions, FSH, LH,

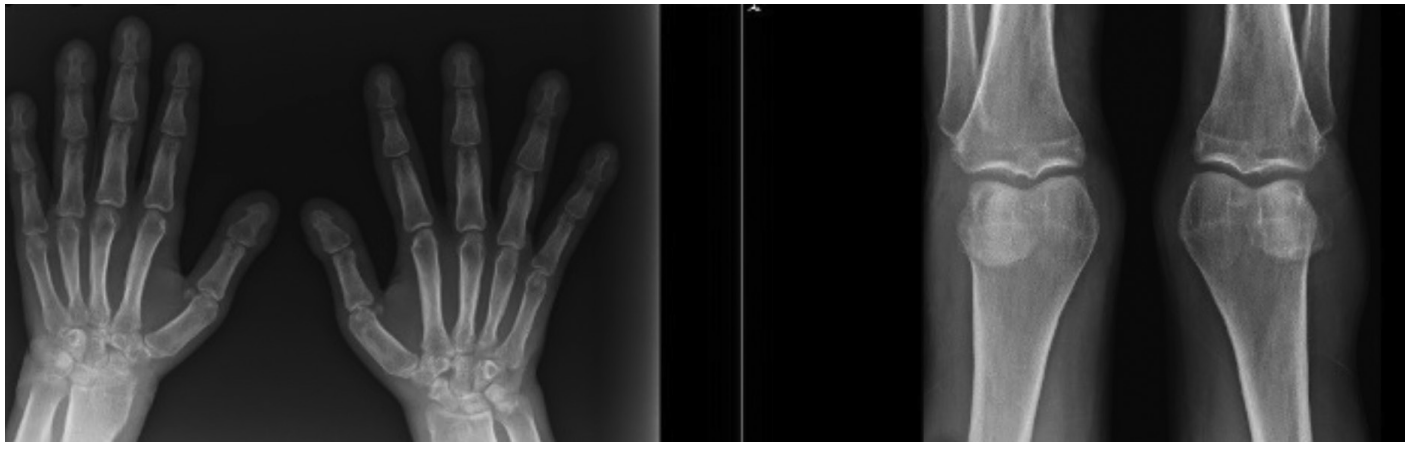

**Figure 2.** Radiography images of the first case

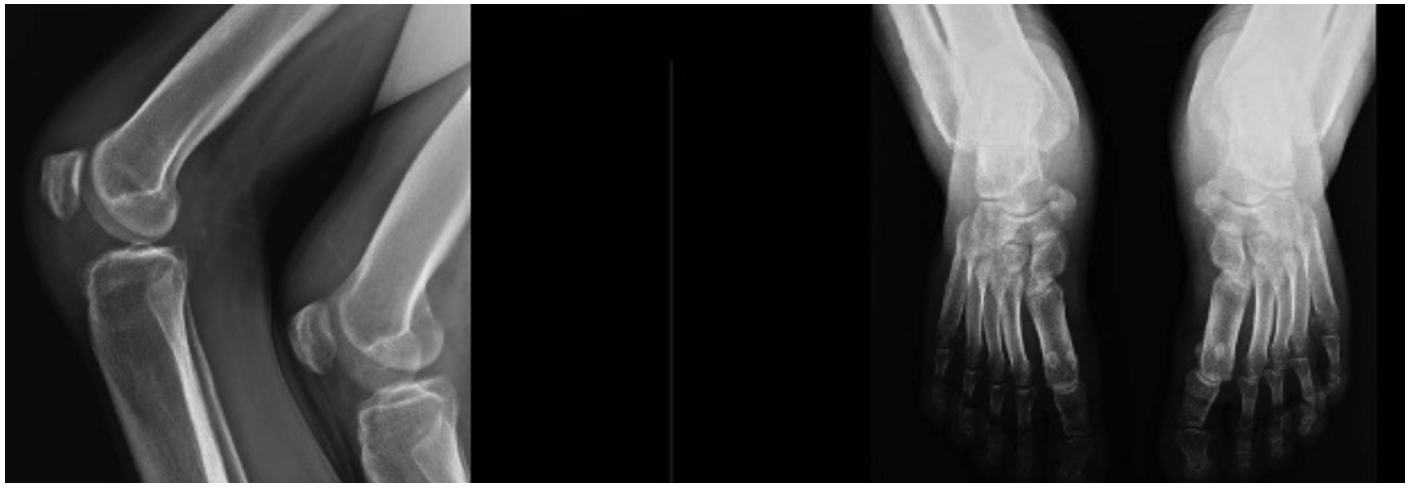

**Figure 3.** Radiography images of the first case

total testosterone, prolactin, rheumatoid factor, and anti-cyclic citrullinated peptide were normal. Serological tests were performed to evaluate connective tissue disorder and vasculitis, which were again all normal. As acromegaly was suspected, IGF-1 and GH measurements were performed and the following values were obtained: IGF-1 311 ng/mL (111-509) and fasting GH 0.45 mcg/L (0.07-5). The OGTT was normal, suppression of GH less than 1 µg/L. Bilateral knee and hand radiographs were considered normal (Figure 5). No pituitary adenoma was detected on pituitary MRI. Chest radiograph and echocardiography were considered normal. No abnormal finding was detected in abdominal and thoracic CT scans. The patient was diagnosed with a rough form of PDP, based on clinical, biochemical, and radiological findings. Genetic analysis revealed a homozygous frameshift variant NM_005630.2:c.86delG (p.G29Afs*48) in exon 1 of the *SLCO2A1* gene. This variant was previously reported in the ClinVar database as pathogenic. Thus, selective Cox-2 inhibitor (meloxicam, 15 mg twice daily, PO) and steroid (methylprednisolone, 5 mg/day, PO) were prescribed. On day six, melena developed in the patient; thus, he underwent esophagogastroduodenoscopy which revealed a duodenal ulcer (2 cm in diameter). The meloxicam and prednisolone therapy were withdrawn and hydroxychloroquine was prescribed (200 mg twice daily, PO). After one month, regression was reported in joint pain and sweating. Marked regression was detected in skin thickening by month six.

**Figure 4.** Physical examination findings of the second case

## Discussion

PDP is a rare hereditary disorder, for which the inheritance pattern has not yet been fully elucidated (6). The first case with PDP was reported by Friedreich in 1868. Touraine, Solente, and Gole defined PDP as a variant of hypertrophic osteoarthropathy, secondary to acromegaly and malignancy (1). As noted, PDP predominantly affects males and both patients reported here were boys (4). PDP begins during childhood or adolescence and progresses gradually over the next 5-20 years, before stabilizing (5). The typical presentations include thickening and coarsening of skin and/or scalp (pachydermia), clubbing of digits, oedema in the lower legs, arthritis both with and without joint effusion, and periostosis (swelling of periarticular tissue and subperiosteal new bone formation). The most common findings are associated with polyarthritis, cutis verticis gyrata, seborrhea and hyperhidrosis. PDP can manifest in three forms: classical or complete form (skin thickening, skeletal changes, and clubbing at fingers), incomplete form

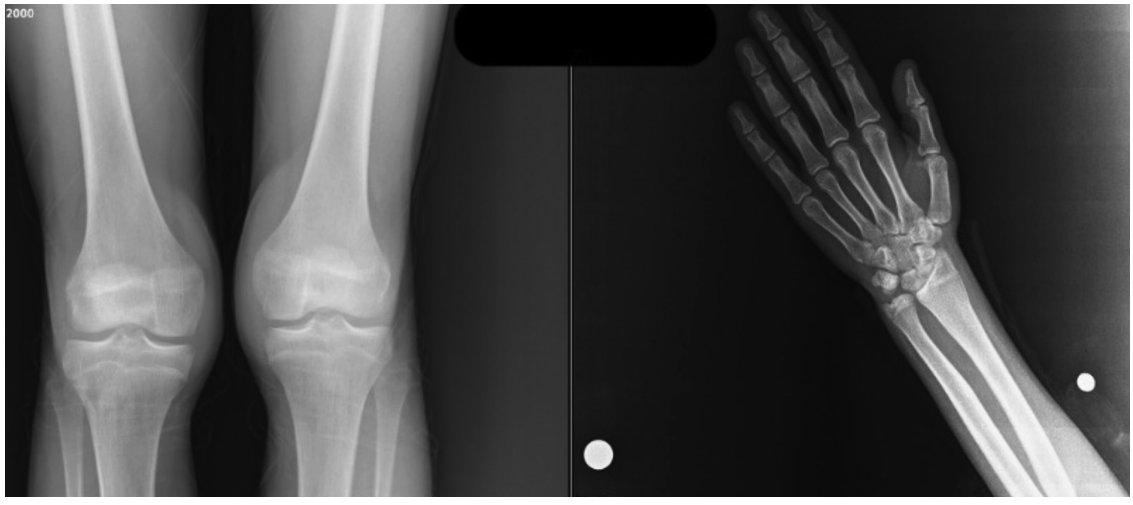

**Figure 5.** Radiography images of the second case

(skeletal changes without skin involvement), and rough form (minimal skeletal change and skin thickening) (1).

PDP is very rare, and its clinical and radiological presentations can be confused with those of acromegaly, pseudoacromegaly with severe insulin resistance, secondary hypertrophic osteoarthropathy, Marfan's syndrome, McCune Albright syndrome, syphilitic periostitis, psoriatic onycho-pachydermo-periostitis and Paget's disease (5,6,7,8,9,10,11). Acromegaly may bedue to sporadic or familial GH-secreting adenomas, arising during childhood or puberty. These features that are present in patients with acromegaly are due to the effect of excess GHs, mainly from pituitary tumours (12). The clinical picture in children and adolescents varies depending on whether the epiphyseal growth plate is open. Before epiphyseal fusion, there is a significant acceleration in growth rate, a condition also known as 'gigantism' but once epiphyseal fusion is complete, clinical symptoms become more similar to those in acromegalic adults, including coarse facial features, broadened nose, large hands and feet, organomegaly, and sweating (13). In acromegaly, excessive GH/IGF-1 production leads to periosteal bone formation, growth of synovial tissue, cartilage and precursor hypertrophic arthropathy associated with pain, and deformity, as seen in PDP. Acral abnormalities associated with PDP may overlap with those seen in acromegaly, including enlarged limbs, enlarged, thickened and short fingers, and thickened soft tissue. While digital clubbing and periostosis are seen in PDP, these findings are not seen in acromegaly (14). Facial coarsening, cutis verticis gyrate, seborrhea, acne and hyperhidrosis, are common in both PDP and in acromegaly. Symptoms specific to PDP but not seen in acromegaly, are long eyelashes, blepharoptosis, myelofibrosis, hypoalbuminemia, peptic ulcer, gastric cancer or watery diarrhoea in response to certain triggers, such as cold drinks, greasy food or sexual activity (15).

The pathogenesis of PDP is not yet clearly understood but evidence suggests that vascular endothelial growth factor (VEGF) and platelet-derived growth factor could play a central role (6). Recent studies demonstrated a prostaglandin mediated pathway as the key player in the pathogenesis of PDP. The 15-hydroxyprostaglandin dehydrogenase (HPGD) enzyme plays an important role in prostaglandin degradation and increased prostaglandin levels, particularly prostaglandin E2 (PGE2) (16). The two major genetic lesions both lead to an increase in PGE2, either by decreased degradation due to enzymatic loss (HPGD mutations) or a transporter defect (with *SLCO2A1* mutations). The genetic assay was performed in both cases in this report, revealing a novel homozygous *SLCO2A1* NM_005630.2: c.31 C>T (p.Q11*) mutation in the first case and a previously reported homozygous *SLCO2A1*NM_005630.2:c.86delG (p.G29Afs*48) mutation in the second case. Radiological findings in PDP include subperiosteal newbone formation, cortical thickening, and narrowing of the joint spaces. Resorption of bone of the distal phalanges and ossification of inter-osseous membranes and ligaments can also be seen (17). In our first case, bilateral hand radiographs revealed periostitis and hyperostosis in the metacarpal and proximal phalanges. In our second case, radiographic examinations were found to be normal.

There is no specific treatment modality for PDP. Both *15 HPGD* and *SLCO2A1* genes are involved in PGE2 synthesis (18,19). COX inhibitors (non-steroidal anti-inflammatory drugs, acetylsalicylic acid and corticosteroid) that inhibit the COX enzyme and suppress PGE2 biosynthesis are promising agents in PDP treatment. We achieved a good response in the first case, but treatment was discontinued in the second case due to upper gastrointestinal bleeding from a duodenal ulcer. In the first case, after six months of meloxicam and prednisolone treatment, the patient's complaints regressed significantly, so prenisolone treatment was discontinued by titration and followed up with meloxicam treatment. Alessandrella et al. (20) observed a marked improvement in skin findings and joint pain with hydroxychloroquine in a PDP patient with a homozygous *SLCO2A1* gene mutation. In the second case in our report, hydroxychloroquine therapy was initiated as a homozygous *SLCO2A1* gene mutation was detected, and marked regression was achieved in skin findings. Other agents used in medical therapy of PDP include aescin, bisphosphonate, colchicine, retinoids, tricyclic antidepressants, and tamoxifen citrate (21,22). Botulinum toxin A has been used for cosmetic reasons. Surgery has been used to correct bone deformity, if present, and plastic surgery can be used to repair thickening in the forehead skin.

When a patient is suspected of having acromegaly, the first step is biochemical testing to confirm the clinical diagnosis, followed by imaging to determine the cause of excessive GH secretion. Pituitary adenoma is present in more than 95 percent of cases (23). The best single test for the diagnosis of acromegaly is measurement of serum IGF-1. Both serum GH concentrations and IGF-1 concentrations are increased in virtually all patients with acromegaly. During OGTT, serum GH level <1 µg/L means the diagnosis of acromegaly is excluded. In PDP patients, IGF-1 and GH are normal and there is no adenoma in the pituitary (20). In both of our patients, GH and IGF-1 were found to be normal and GH after OGTT was <1 ng/mL while pituitary MRI reported no adenoma. Although transsphenoidal surgery is recommended as the first step in treatment, treatment with

a long-acting somatostatin analogue is also used in cases that do not respond to surgery (24).

## Conclusion

In conclusion, the clinical presentation of PDP can be confused with multiple other diagnoses, especially acromegaly. Therefore PDP should be considered in the differential diagnosis of individuals presenting with acromegaloid feature.

### Ethics

**Ethics Committee Approval:** All procedures performed during this retrospective study were following the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical committee approval is not required for case reports.

**Informed Consent:** Informed consent forms were obtained from the patients and their families.

### Authorship Contributions

Surgical and Medical Practices: Emine Kartal Baykan, Ayberk Türkyılmaz, Concept: Emine Kartal Baykan, Ayberk Türkyılmaz, Design: Emine Kartal Baykan, Ayberk Türkyılmaz, Data Collection or Processing: Emine Kartal Baykan, Ayberk Türkyılmaz, Analysis or Interpretation: Emine Kartal Baykan, Ayberk Türkyılmaz, Literature Search: Emine Kartal Baykan, Ayberk Türkyılmaz, Writing: Emine Kartal Baykan.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Thomas RH, Kirby JD. Pachydermoperiostosis with multiple basal cell carcinomata. J R Soc Med 1985;78:335-337.
2. Salah BI, Husari KI, Hassouneh A, Al-Ali Z, Rawashdeh B. Complete primary pachydermoperiostosis: A case report from Jordan and review of literature. Clin Case Rep 2019;7:346-352.
3. Doshi D. Touraine-Solente-Gole syndrome. Orbit 2018;37:97-101. Epub 2017 Oct 17
4. Reginato AJ, Schiapachasse V, Guerrero R. Familial idiopathic hypertrophic osteoarthropathy and cranial suture defects in children. Skeletal Radiol 1982;8:105-109.
5. Abdullah NRA, Jason WLC, Nasruddin AB. Pachydermoperiostosis: a rare mimicker of acromegaly. Endocrinol Diabetes Metab Case Rep 2017;2017:17-0029.
6. Uppal S, Diggle CP, Carr IM, Fishwick CW, Ahmed M, Ibrahim GH, Helliwell PS, Latos-Bieleńska A, Phillips SE, Markham AF, Bennett CP, Bonthron DT. Mutations in 15-hydroxyprostaglandin dehydrogenase cause primary hypertrophic osteoarthropathy. Nat Genet 2008;40:789-793.
7. Berlin L. Duke Radiology Case Review: Imaging, Differential Diagnosis, and Discussion. JAMA 2012;307:2199-2200.
8. Boisseau-Garsaud AM, Beylot-Barry M, Doutre MS, Beylot C, Baran R. Psoriatic onycho-pachydermo-periostitis. A variant of psoriatic distal interphalangeal arthritis? Arch Dermatol 1996;132:176-180.
9. Auger DM, Stavrianeas N. Pachydermoperiostosis Member of The European Editorial Committee of Orphanet Encyclopedia, 2004.
10. Chakraborty RK, Sharma S. Secondary Hypertrophic Osteoarthropathy. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2020.
11. Ulutaş F, Ulu M, Karasu U, Çobankara V. Hypertrophic Osteoarthropathy Presenting as Rheumatoid Arthritis Mimicker: A Case Report. Cureus 2020;12:e9271.
12. Pandey P, Ojha BK, Mahapatra AK. Pediatric pituitary adenoma: A series of 42 patients. J Clin Neurosci 2005;12:124-127.
13. Laws ER Jr, Scheithauer BW, Carpenter S, Randall RV, Abboud CF. The pathogenesis of acromegaly. Clinical and immunocytochemical analysis in 75 patients. J Neurosurg 1985;63:35-38.
14. Pineda CJ, Martinez-Lavin M, Goobar JE, Sartoris DJ, Clopton P, Resnick D. Periostitis in hypertrophic osteoarthropathy: relationship to disease duration. AJR Am J Roentgenol 1987;148:773-778.
15. Marques P, Stelmachowska-Banas M, Collier D, Wernig F, Korbonits M. Pachydermoperiostosis mimicking the acral abnormalities of acromegaly. Endocrine 2020;67:499-500. Epub 2020 Jan 8
16. Tanese K, Niizeki H, Seki A, Otsuka A, Kabashima K, Kosaki K, Kuwahara M, Miyakawa S, Miyasaka M, Matsuoka K, Okuyama T, Shiohama A, Sasaki T, Kudoh J, Amagai M, Ishiko A. Pathological characterization of pachydermia in pachydermoperiostosis. J Dermatol 2015;42:710-714.
17. Mangupli R, Daly AF, Cuauro E, Camperos P, Krivoy J, Beckers A. Primary hypertrophic osteoarthropathy due to a novel SLCO2A1 mutation masquerading as acromegaly. Endocrinol Diabetes Metab Case Rep 2017;2017:17-0013.
18. Nakanishi T, Nakamura Y, Umeno J. Recent advances in studies of SLCO2A1 as a key regulator of the delivery of prostaglandins to their sites of action. Pharmacol Ther 2021;223:107803. Epub 2021 Jan 16
19. Kozak KR, Milne GL, Morrow JD, Cuiffo BP. Hypertrophic osteoarthropathy pathogenesis: A case highlighting the potential role for cyclo-oxygenase-2-derived prostaglandin E2. Nat Clin Pract Rheumatol 2006;2:452-456.
20. Alessandrella A, Della Casa R, Alessio M, Puente Prieto J, Strisciuglio P, Melis D. A novel homozygous mutation in the SLCO2A1 gene causing pachydermoperiostosis: Efficacy of hydroxychloroquine treatment. Am J Med Genet A 2018;176:1253-1257.
21. 21 Zhang H, Yang B. Successful treatment of pachydermoperiostosis patients with etoricoxib, aescin, and arthroscopic synovectomy: Two case reports. Medicine (Baltimore) 2017;96:e8865.
22. Chakraborty PP, Bhattacharjee R, Mukhopadhyay S, Chowdhury S. Pseudoacromegaly in pachydermoperiostosis. BMJ Case Rep 2016;2016:bcr2016214624.
23. Melmed S. Medical progress: Acromegaly. N Engl J Med 2006;355:2558-2573. Erratum in: N Engl J Med 2007;356:879.
24. Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A, Wass JA; Endocrine Society. Acromegaly: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2014;99:3933-3951. Epub 2014 Oct 30

CASE REPORT

DOI: 10.4274/jcrpe.galenos.2021.2020.0273

J Clin Res Pediatr Endocrinol 2022;14(3):356-360

# Central Precocious Puberty in an Infant with Sotos Syndrome and Response to Treatment

**Tuğba Kontbay[1], Zeynep Şıklar[2], Serdar Ceylaner[3], Merih Berberoğlu[2]**

*[1]Şanlıurfa Training and Research Hospital, Clinic of Pediatric Endocrinology, Şanlıurfa, Turkey*
*[2]Ankara University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey*
*[3]Intergen Genetic Center, Ankara, Turkey*

**What is already known on this topic?**

Sotos syndrome (SS) is characterized by overgrowth, typical facial appearance, and learning disability. While advanced bone age can be detected in some cases, precocious puberty (PP) has only been reported in three cases previously.

**What this study adds?**

In some specific syndromes with PP, such as SS, treatment can be difficult. Maximum dose gonadotropin releasing hormone analog may not be successful for the control of pubertal progression. Cyproterone acetate may be beneficial for treatment.

## Abstract

Sotos syndrome (SS) is characterized by overgrowth, distinctive facial appearance, and learning disability. It is caused by heterozygous mutations, including deletions of *NSD1* located at chromosome 5q35. While advanced bone age can occur in some cases, precocious puberty (PP) has only been reported in three cases previously. Here, we reported a case of SS diagnosed in the infancy period with central PP. The discovery of potential factors that trigger puberty is one of the central mysteries of pubertal biology. Depot gonadotropin-releasing hormone analogs constitute the first-line therapy in central PP (CPP), which has proven to be both effective and safe. In our cases, leuprolide acetate at maximum dose was not successful in controlling pubertal progression, and cyproterone acetate (CPA) was added to therapy, with successful control of pubertal progression. In some specific syndromes with PP, such as SS, treatment can be challenging. CPA may be an asset for effective treatment.

**Keywords:** Sotos syndrome, precocious puberty, NSD1, cyproterone acetate

## Introduction

Sotos syndrome (SS) is a rare syndrome with an incidence of approximately 1 in 14,000 live births (1). This childhood overgrowth syndrome of prenatal onset is characterized by increased birth length or head circumference, typically greater than two standard deviations from the mean, distinctive facial features, excessive growth during the first four years, and advanced bone age. Children with SS have macrodolichocephaly, broad and prominent forehead, and bitemporal narrowing (2). Also, they may present with neonatal hypotonia, delays in motor development and intellectual disabilities (2). The majority of cases have a mutation in the nuclear receptor-binding SET domain-containing protein 1 gene (*NSD1*), which are mostly *de novo* (3). SS results from loss of function mutations, primarily truncating mutations, and whole gene deletions (1,4,5). The four major diagnostic criteria were based on the systematic assessment of 41 typical cases and include overgrowth with advanced bone age, macrocephaly, distinctive facial appearance, and learning difficulties. Features of this syndrome were re-evaluated after the identification of *NSD1* mutations, and the Childhood Overgrowth Collaboration Consortium reviewed the clinical features of cases with *NSD1* abnormalities (6).

Several endocrine problems may occur in SS (7). While advanced bone age can be detected in some cases,

**Address for Correspondence:** Tuğba Kontbay MD, Şanlıurfa Training and Research Hospital, Clinic of Pediatric Endocrinology, Şanlıurfa, Turkey
**Phone:** +90 537 408 83 10 **E-mail:** tugbakontbay@gmail.com **ORCID:** orcid.org/0000-0001-7702-8296

**Conflict of interest:** None declared
**Received:** 16.11.2020
**Accepted:** 07.05.2021

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

precocious puberty (PP) has only been reported in three cases until now, and the reason for PP remains unknown (8,9,10). Also, long term follow-up characteristics and response to treatment had not been discussed in the literature. Here, we reported a case of SS associated with central PP (CPP) and the difficulties of clinical management.

## Case Report

A 6.5 month-old male infant presented with neuromotor delay and macrogenitalia. He was the second child of non-related parents, and his birth-weight was 4200 g. There were no other pathological features on physical examination, except for height which was 81 cm (SDS +5.6), testicular volumes of 4 mL bilaterally, and penis length of 6.7 cm. Mild facial dysmorphism with global developmental delay was noted. High basal testosterone level (88 ng/dL), and high basal and stimulated gonadotropins (basal luteinizing hormone [LH] 1.56 mIU/mL, basal follicle-stimulating hormone [FSH] 1.02 mIU/mL, stimulated LH 43.3 mIU/mL, and stimulated FSH 3.65 mIU/mL) confirmed the CPP. Cranial imaging studies revealed normal pituitary gland with dilated perivascular cavities in subcortical white matter, dilatation of cerebral lateral ventricles, and cavum septum pellucidum. The patient's bone age was found to be one-year-old.

Gonadotropin releasing hormone (GnRH) analog at a dose of 250 mcg/kg/month was started. Due to inefficient hypothalamic-pituitary-gonadal (HHG) axis control, the GnRH analog dose was increased to 500 mcg/kg/month. At the age of 2.5 years an increase of testicular volume to 8 mL and penile length to 9 cm was observed, and his bone age had advanced to 4.5 years after 20 months of treatment with GnRH analog. The basal LH level was 1.6 mIU/mL, and the basal testosterone level 58 ng/dL. Due to continued pubertal progression and unsuccessful suppression of LH and testosterone level, cyproterone acetate (CPA) 50 mg/day was added to his treatment. Over the next three years with combined treatment, the patient's clinical and laboratory progression was controlled. At the last examination, he was 6.24 years old, and his height was 139 cm [standard deviation score (SDS) +4.52]. The patient's testicular volume regressed to 5 mL, and basal testosterone level was suppressed to prepubertal level. Laboratory results of the patient before and during treatment are shown in Table 1.

As his phenotype resembled SS, *NSD1* analysis was performed, and a heterozygous mutation NM_022455.4: c.5177C>G (p.Pro1726Arg) was detected. This variant was not found in gnomAD exomes and gnomAD genomes. There are several other pathogenic mutations very near this codon in this gene. Pathogenic computational results were based on 12 pathogenic predictions from BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT versus no benign predictions. ClinVar classified this variant as Pathogenic. Thus this variant was evaluated as pathogenic due to the American College of Medical Genetics criteria. Informed consent was received from his parents.

## Discussion

SS is characterized by overgrowth and typical facial features, but other clinical features and their molecular bases have been identified as well. Additional features may be present, potentially as a consequence of micro-deletions encompassing other genes in addition to *NSD1* (11,12). Our patient was large for his gestational age (birth weight was 4200 g, gestation age was normal). He had distinctive facial features with macrodolichocephaly, marked frontal bossing, with a long and thin face in addition to global developmental delay. While CPP was the most striking feature of the patient, other clinical characteristics were compatible with SS. He was clinically considered SS, then *NSD1* analysis was performed, and a heterozygous mutation NM_022455.4: c.5177C>G (p.Pro1726Arg) was detected.

Many benign and pathogenic variants of *NSD1* have been identified. However, the mechanismof functional abrogation

**Table 1. Laboratory results of the patient before and during treatment**

| Age (year) | Basal LH (mIU/mL) | Basal FSH (mIU/mL) | Testosterone (ng/dL) | Peak LH (mIU/mL) | Peak FSH (mIU/mL) | Treatment |
|---|---|---|---|---|---|---|
| 0.6 | 1.02 | 1.56 | 88 | 43.3 | 3.65 | GnRH a (250 mcg/kg/month) |
| 1.28 | 3.52 | 0.39 | 67 | 6 | 0.54 | GnRH a (500 mcg/kg/month) |
| 2.5 | 1.61 | 0.13 | 58 | 6.5 | 0.53 | GnRH a (500 mcg/kg/month) + Cyproterone acetate |
| 6.24 | 1.2 | 0.2 | <10 | 3.1 | 0.36 | GnRH a (500 mcg/kg/month) + Cyproterone acetate |

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

of *NSD1* resulting in SS remains unknown. It is thought that there may be a link between SS and rat sarcoma-mitogen-activated protein kinase (RAS-MAPK) signaling pathway, which is downregulated in SS (13). RAS-MAPK pathway is also known as the Ras-Raf-MEK-ERK (MAPK/ERK). However, Ras interacting protein 1, a downstream Ras effector interfering with the MAPK/ERK pathway, is identified as being upregulated in SS (14). The deregulation of the MAPK/ERK-signaling cascade causes a hypertrophic differentiation of *NSD1*-expressing chondrocytes with subsequent statural overgrowth and accelerated skeletal maturation in patients with SS (14).

Our case presented with CPP at a very early age. CPP in SS is an infrequent finding, although some endocrine problems such as hypoglycemia, hypothyroidism, hypospadias, and cryptorchidism in infancy have been reported (7).

Cases such as ours, caused by central activation of the HHG axis are referred to as CPP, the etiology of which can be idiopathic, familial, or secondary to structural brain anomalies (15,16). CPP may have critical underlying causes, including acquired and congenital central nervous system (CNS) lesions or congenital causes without CNS lesions, such as complex syndromic phenotypes with or without known chromosomal abnormalities or genetic changes. Most of the time, the common cause of CPP in females is idiopathic, while in males, there is usually an underlying pathology. CPP may develop in infancy in male patients with an organic lesion (17).

For SS, only three cases with PP have been reported to date in the literature (8,9,10). The first case with CPP of SS was reported in 1995. Although we could not obtain detailed information about this case, she was reported to have premature pubarche and premature pubertal development (8). The second case was presented at the European Society for Paediatric Endocrinology meeting in 2016. He was diagnosed at 6.8 years of age with CPP with a global developmental delay. *NSD1* gene deletion was found after the diagnosis of PP. This case had normal pituitary and brain magnetic resonance imaging (MRI) (9). The third case was a 3-month-old boy, reported by Gupta and Dayal (10), who presented with enlargement of genitalia and rapid growth noticed since birth and was diagnosed with CPP and SS. Genetic analysis identified a pathogenic heterozygous mutation in the *NSD1* gene (c.2362C > T; p. Arg788Ter).

The pathogenesis of CPP is not yet fully understood in this syndrome. Some structural brain abnormalities, which usually develop in SS, might induce CPP by activating the HHG axis. In our patient, cranial MRI revealed brain formation anomalies (dilatation of cerebral lateral ventricles and cavum septum pellucidum), but the hypothalamus and hypophysis were normal. However, the cause of CPP may not be related to such cranial structural abnormalities since not all SS cases have CPP despite having similar cranial imaging features. A disorder of the central activation of the HHG axis is likely, although the underlying pathophysiological mechanism is not yet determined (13).

Many factors that regulate the timing of puberty remains unclear, despite recent advances. Some syndromes associated with disorders of pubertal timing provide opportunities to identify genetic regulation of puberty. RASopathies are developmental disorders caused by heterozygous activating germline mutations in RAS-MAPK pathway genes. The RAS-MAPK pathway, also known as the Ras-Raf-MEK-ERK pathway, plays a central role in signal transduction from extracellular stimuli to the intracellular environment (18,19). The RAS-MAPK pathway is one of the pathways involved in the regulation of the GnRH receptor signaling cascades. GnRH receptor signaling results in the secretion of LH and FSH. Therefore, genetic abnormalities in this pathway could hypothetically lead to either delayed or PP development. Although, as with PP, this association's exact etiology is not fully understood (20). It can be suggested that CPP in SS may be associated with these RAS-MAPK signaling pathways and *NSD1* gene relationships. Due to this unusual clinical condition, it is important to report such cases and find common points to help understand the etiology. Besides, future comprehensive studies of pubertal development in patients with SS will help to explore the pathophysiological relevance of mechanisms underlying the precocious onset of puberty in these disorders.

Although some cases have been challenging to manage with standard doses, GnRH analog treatment effectively controls CPP in most cases (21). In our case, GnRH analog treatment at high doses could not effectively control the CPP, so CPA was added to therapy. CPAs have both anti-gonadotropic and anti-androgenic features. In addition to blocking the GnRH analogs initial stimulatory effect on the pituitary somatotrophs, CPA has anti-androgenic activity, partly due to its adrenocorticotropic hormone suppressing activity but also to a direct antiandrogen effect (22). Over 20 months of treatment with GnRH analog, the patient's basal LH was 1.6 mIU/mL, basal testosterone level 58 ng/dL, and his bone age advanced to 4.5 years. Due to this pubertal progression and unsuccessful suppression of the LH-testosterone level, CPA 50 mg/day was added to treatment. CPA is a beneficial therapy in CPP therapy with gonadotropin suppressing and

androgen inhibiting effects, which was not controlled by standard GnRH analog treatment.

Over the next three years with combined treatment, the patient's clinical and laboratory progression was finally controlled. CPA may have adverse effects, mainly in adults and with high doses (20). We found no alterations in liver function tests, despite routine checking, during three years of experience with CPA.

## Conclusion

In conclusion, CPP can very rarely accompany SS, and overgrowth can be related either to the SS itself or the PP. Treatment can also be very challenging, requiring high dose GnRH analog and possibly the addition of CPA. Also, The addition of CPA in this case helped control pubertal progression. Although the etiology of CPP in SS is unknown, it may be related to mutation characteristics of *NDS1* or other underlying reasons that will require further clinical evidence.

### Ethics

**Informed Consent:** Informed consent was received from his parents.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: Tuğba Kontbay, Zeynep Şıklar, Merih Berberoğlu, Design: Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Tuğba Kontbay, Zeynep Şıklar, Analysis or Interpretation: Tuğba Kontbay, Zeynep Şıklar, Serdar Ceylaner, Merih Berberoğlu, Literature Search: Tuğba Kontbay, Zeynep Şıklar, Writing: Tuğba Kontbay, Zeynep Şıklar, Serdar Ceylaner.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Tatton-Brown K, Douglas J, Coleman K, Baujat G, Cole TR, Das S, Horn D, Hughes HE, Temple IK, Faravelli F, Waggoner D, Turkmen S, Cormier-Daire V, Irrthum A, Rahman N; Childhood Overgrowth Collaboration. Genotype-phenotype associations in Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations. Am J Hum Genet 2005;77:193-204. Epub 2005 Jun 7
2. Edmondson AC, Kalish JM. Overgrowth Syndromes. J Pediatr Genet 2015;4:136-143. Epub 2015 Sep 25
3. Tatton-Brown K, Douglas J, Coleman K, Baujat G, Chandler K, Clarke A, Collins A, Davies S, Faravelli F, Firth H, Garrett C, Hughes H, Kerr B, Liebelt J, Reardon W, Schaefer GB, Splitt M, Temple IK, Waggoner D, Weaver DD, Wilson L, Cole T, Cormier-Daire V, Irrthum A, Rahman N; Childhood Overgrowth Collaboration. Multiple mechanisms are implicated in the generation of 5q35 microdeletions in Sotos syndrome. J Med Genet 2005;42:307-313.
4. Kurotaki N, Imaizumi K, Harada N, Masuno M, Kondoh T, Nagai T, Ohashi H, Naritomi K, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Hasegawa T, Chinen Y, Tomita Ha HA, Kinoshita A, Mizuguchi T, Yoshiura Ki K, Ohta T, Kishino T, Fukushima Y, Niikawa N, Matsumoto N. Haploinsufficiency of NSD1 causes Sotos syndrome. Nat Genet 2002;30:365-366. Epub 2002 Mar 18
5. Kamimura J, Endo Y, Kurotaki N, Kinoshita A, Miyake N, Shimokawa O, Harada N, Visser R, Ohashi H, Miyakawa K, Gerritsen J, Innes AM, Lagace L, Frydman M, Okamoto N, Puttinger R, Raskin S, Resic B, Culic V, Yoshiura K, Ohta T, Kishino T, Ishikawa M, Niikawa N, Matsumoto N. Identification of eight novel NSD1 mutations in Sotos syndrome. J Med Genet 2003;40:e126.
6. Baujat G, Cormier-Daire V. Sotos syndrome. Orphanet J Rare Dis 2007;2:36.
7. Visser R, Landman EB, Goeman J, Wit JM, Karperien M. Sotos syndrome is associated with deregulation of the MAPK/ERK-signaling pathway. PLoS One 2012;7:e49229. Epub 2012 Nov 14
8. Bertelloni S, Baroncelli GI, Tomasi O, Sorrentino MC, Costa S, Saggese G. Sindrome di Sotos: follow-up di un caso con pubertà anticipata [Sotos syndrome: follow-up of a case with precocious puberty]. Pediatr Med Chir 1995;17:353-357.
9. Lim S. Central precocious puberty in a case of SOTOS syndrome (abstract). In: 53rd Annual Meeting of the European Society for Paediatric Endocrinology (ESPE). Dublin, Ireland, September 18-20, 2014: Horm Res Pediatr 2014;82:1-507.
10. Gupta S, Dayal D. Precocious Puberty in an Infant with Sotos Syndrome. Indian Pediatr 2019;56:516.
11. Nagai T, Matsumoto N, Kurotaki N, Harada N, Niikawa N, Ogata T, Imaizumi K, Kurosawa K, Kondoh T, Ohashi H, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Yokoyama T, Uetake K, Sakazume S, Fukushima Y, Naritomi K. Sotos syndrome and haploinsufficiency of NSD1: clinical features of intragenic mutations and submicroscopic deletions. J Med Genet 2003;40:285-289.
12. Niikawa N. Molecular basis of Sotos syndrome. Horm Res 2004;62 Suppl 3:60-65.
13. Wannes S, Elmaleh-Bergès M, Simon D, Zénaty D, Martinerie L, Storey C, Gelwane G, Paulsen A, Ecosse E, De Roux N, Carel JC, Léger J. High prevalence of syndromic disorders in patients with non-isolated central precocious puberty. Eur J Endocrinol 2018;179:373-380.
14. Laccetta G, Moscuzza F, Michelucci A, Guzzetta A, Lunardi S, Lorenzoni F, Ghirri P. A Novel Missense Mutation of the NSD1 Gene Associated with Overgrowth in Three Generations of an Italian Family: Case Report, Differential Diagnosis, and Review of Mutations of NSD1 Gene in Familial Sotos Syndrome. Front Pediatr 2017;5:236.
15. Argyropoulou MI, Kiortsis DN. MRI of the hypothalamic-pituitary axis in children. Pediatr Radiol 2005;35:1045-1055. Epub 2005 Jun 1
16. Mogensen SS, Aksglaede L, Mouritsen A, Sørensen K, Main KM, Gideon P, Juul A. Pathological and incidental findings on brain MRI in a single-center study of 229 consecutive girls with early or precocious puberty. PLoS One 2012;7:e29829. Epub 2012 Jan 12
17. Kota AS, Ejaz S. Precocious Puberty. 2019 Nov 18. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
18. Tidyman WE, Rauen KA. Noonan, Costello and cardio-facio-cutaneous syndromes: dysregulation of the Ras-MAPK pathway. Expert Rev Mol Med 2008;10:e37.
19. Zenker M. Clinical manifestations of mutations in RAS and related intracellular signal transduction factors. Curr Opin Pediatr 2011;23:443-451.

20. van der Kaay DC, Levine BS, Doyle D, Mendoza-Londono R, Palmert MR. RASopathies Are Associated With Delayed Puberty; Are They Associated With Precocious Puberty Too? Pediatrics 2016;138:e20160182.

21. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR; ESPE-LWPES GnRH Analogs Consensus Conference Group, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. Pediatrics 2009;123:e752-e762. Epub 2009 Mar 30

22. Laron Z, Kauli R. Experience with cyproterone acetate in the treatment of precocious puberty. J Pediatr Endocrinol Metab 2000;13 Suppl 1:805-810.

CASE REPORT

DOI: 10.4274/jcrpe.galenos.2021.2021.0005

J Clin Res Pediatr Endocrinol 2022;14(3):361-365

# Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked Syndrome in Two Siblings: Same Mutation But Different Clinical Manifestations at Onset

**Gülay Karagüzel[1], Recep Polat[1], Mehtap H. Abul[2], Alper Han Cebi[3], Fazıl Orhan[2]**

*[1]Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Turkey*
*[2]Karadeniz Technical University Faculty of Medicine, Department Pediatric Allergy and Immunology, Trabzon, Turkey*
*[3]Karadeniz Technical University Faculty of Medicine, Department of Genetics, Trabzon, Turkey*

**What is already known on this topic?**

Though enteropathy, endocrinopathy and skin manifestations are considered the classic triad immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, it is clinically characterized by a wide spectrum of severe autoimmune diseases. The absence of the classic clinical triad of the disease may lead to a delay in diagnosis of IPEX syndrome.

**What this study adds?**

IPEX syndrome presented with different clinical pictures in two siblings, despite having the same *FOXP3* mutation. IPEX syndrome should be considered in any male infant with only one main disorder typical of IPEX syndrome, such as infantile diabetes, and not only in infants with the full classical triad.

## Abstract

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is an early onset systemic autoimmune genetic disorder caused by mutation of the *forkhead box protein 3 (FOXP3)* gene. Enteropathy, endocrinopathy and skin manifestations are considered the classic triad of IPEX syndrome. However, patients with IPEX syndrome display a variety of phenotypes including life threatening multi-organ autoimmunity. Here, we present the case of two siblings with IPEX syndrome with the same hemizygous mutation, but with different types of symptomology at onset of the disease.

**Keywords:** Immune dysregulation polyendocrinopathy enteropathy X-linked syndrome, neonatal diabetes, renal disease

## Introduction

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is an early onset, systemic autoimmune genetic disorder caused by mutation of the *forkhead box protein 3 (FOXP3)* gene. *FOXP3* is located in the short arm of the X chromosome (Xp11.3-q13.3). The *FOXP3* gene regulates production and function of the FOXP3 protein which is essential for the regulatory function of T regulatory ($T_{reg}$) cells (1,2). $T_{reg}$ suppress a range of functions of neighboring T effector ($T_{eff}$) cells. $T_{reg}$ cell dysfunction is the main cause of immune dysfunction in IPEX syndrome, including severe enteropathy, type 1 diabetes, and dermatitis. However, persistent autoreactive B cells and autoreactive $T_H17$ cell expansion contribute to autoimmune disorder in this syndrome (1,2,3). Recurrent infections, hemolytic anemia and cytopenia, autoimmune thyroiditis, and other autoimmune disorders, such as hepatitis and nephritis, may be additional manifestations of IPEX syndrome (3).

IPEX syndrome is clinically characterized by a wide spectrum of severe autoimmune diseases, but patients with IPEX syndrome commonly present with early onset, intractable diarrhea within the first weeks of life. If not treated, most

**Address for Correspondence:** Gülay Karagüzel MD, Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Turkey
**Phone:** +90 542 425 69 56 **E-mail:** gulkarag@hotmail.com, gkaraguzel@ktu.edu.tr
**ORCID:** orcid.org/0000-0003-4116-5365

**Conflict of interest:** None declared
**Received:** 06.01.2021
**Accepted:** 10.05.2021

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*The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.*

patients die within the first two years of life because of the consequences of autoimmune manifestations, sepsis, and complications from failure to thrive (2). Nevertheless, there are some reports of patients with longer life. The type and severity of symptoms and the onset of the IPEX syndrome may differ from one patient to another, causing diagnostic delays (3,4,5,6,7).

Here, we present two siblings with IPEX syndrome, with the same hemizygous mutation, but with different types of symptomology at onset; one with dermatitis, type 1 diabetes mellitus and autoimmune hemolytic anemia (AHA) while the other presented with dermatitis, enteritis, and glomerulonephritis. Verbal and written parental informed consents were obtained for both siblings.

## Case Reports

### Case 1

A 5-month old male infant was referred for evaluation for hyperglycemia. His past history and medical records revealed a two-week period of hospitalization at 3-month of age with bronchopneumonia that was treated with antibiotics, and hyperglycemia with normal hemoglobin A1c (HbA1c) and C-peptide levels that was controlled by insulin within three days. According to his family history, this patient was the fourth child of nonconsanguineous, healthy parents and had two healthy elder sisters and one elder brother (case 2) with diagnoses of mesangial proliferative glomerulonephritis, and suspected immune deficiency which was not definitively diagnosed due to the presence of malnutrition, chronic diarrhea, dermatitis, elevated total immunoglobulin E (IgE) levels and recurrent severe respiratory tract infections.

Physical examination at admission revealed a pale infant with eczematous lesions of the neck and scalp. His weight and height were at the fiftieth percentile (7.3 kg and 66.5 cm, respectively). The findings from the rest of his physical examination were unremarkable.

His main clinical and laboratory findings at admission are shown in Table 1. Presence of an elevated reticulocyte count (4.49%), a positive result of a direct Coombs test, and peripheral blood smear showing hemolysis, suggested the diagnosis of AHA. Serum chemical analyses showed an elevated serum glucose with normal blood gas analysis. Urine analysis was normal except for glucosuria. His HbA1c was slightly elevated with reduced and normal fasting and postprandial C-peptide levels (0.68 ng/mL and 1.86 ng/mL, respectively, normal range 0.9-7.1 ng/mL). Anti-islet cell and anti-insulin antibodies were normal, whereas anti-glutamic acid decarboxylase was elevated. He was diagnosed as diabetes mellitus and insulin treatment was started.

Having infantile diabetes mellitus, dermatitis and AHA, and an elder brother with suspected immune deficiency, IPEX syndrome was considered. The diagnosis was established genetically by *FOXP3* whole gene sequence analysis that revealed a hemizygous p.250K.del (c.748_750delAAG) mutation. His mother was found to be heterozygous for the same mutation. The same mutation has been reported in two previous cases with IPEX syndrome (7,8).

At the age of 1 year the patient underwent a successful hematopoietic, HLA-matched, sibling donor stem cell transplantation (HSCT) with significant improvement in his general course. Dermatitis, and AHA completely resolved at 14 months of age. However, after a 3-month insulin-free period, hyperglycemia recurred and a course of insulin treatment had to be restarted. At the last follow-up at the age of 4.6 years, the patient was in good clinical condition with normal growth and HbA1c has been maintained at $<8$ % with insulin (0.8 IU/kg/day). There are also no accompanying endocrine diseases, with the exception of diabetes mellitus.

### Case 2

This patient is the elder brother of case 1 and the third child of the parents. At the age of 7 months, he was diagnosed with eczematous dermatitis due to skin lesions localized on the neck and trunk. He developed intractable diarrhea and albuminuria when he was around one year old. After that, he was hospitalized several times in different medical centers due to recurrent pneumonia with eczematous skin lesions or albuminuria diagnosed as mesangial proliferative glomerulonephritis by renal biopsy at the age of 18 months. IgE level was as high as 1368 U/mL, while other immunoglobulins were normal (Table 1). Afterwards, he was suspected of having an immune deficiency, as he had malnutrition, chronic diarrhea, dermatitis, elevated total immunoglobulin E levels and recurrent severe respiratory tract infections but this could not be definitively diagnosed. His CD4 and CD8 T lymphocyte counts, NK cells and CD19 B lymphocyte counts were within normal limits. Isohemagglutinins could not be investigated since his blood group was AB. He had a positive response againts hepatitis B vaccine but pneumococcal vaccine response was not investigated as the testing was not available. The patient had lifelong problems with mesangial proliferative glomerulonephritis requiring immunosuppression with steroids. After we diagnosed his younger brother (case 1) as IPEX syndrome, the same diagnosis for case 2 was thought probable and was subsequently confirmed by showing the same mutation [hemizygous p.250delK (c.748_750delAAG)]

in exon 6 of *FOXP3* when he was 3.5 years old. The patient died of candida sepsis following severe pneumonia at the age of 4 years. No evidence of diabetes mellitus or other endocrine disease was detected in this patient during his lifetime.

## Discussion

Enteropathy, endocrinopathy and skin manifestations are considered the classic triad of IPEX syndrome (2,4,5). However, patients with IPEX syndrome display a variety of phenotypes, including life threatening multi-organ autoimmunity. We report two siblings with IPEX syndrome, who presented with different clinical manifestations in the infantile period despite having the same mutation in the *FOXP3* gene. In addition to dermatitis and immune deficiency findings with recurrent infections, one of the siblings had diabetes mellitus and AHA without enteropathy and the other had mesangial proliferative glomerulonephritis with enteropathy in the first year of the life.

Autoimmune enteropathy is the most common manifestation of IPEX syndrome which present within the early weeks of life with intractable diarrhea. Case 1 had never experienced chronic diarrhea up to last follow up, but case 2 had intractable diarrhea around the age of one.

It has been reported that the frequency of autoimmune endocrinopathies among IPEX patients is 65%, which most commonly include type 1 diabetes and hypothyroidism or hyperthyroidism (3,5). Moreover, it has been suggested that some of the cases of infantile diabetes of unknown etiology might be a mild form of IPEX syndrome (9). Bae et al. (10) reported an IPEX patient who presented with diabetes at 11 months of age but the diagnosis of IPEX was made 10 years later. Therefore, the diagnosis of IPEX should be kept

**Table 1. The clinical and laboratuary features of the presented two siblings and other reported IPEX patients with the same mutation**

| | Case 1 at diagnosis | Case 2 at onset/diagnosis | Hashimura et al. (7) | Wildin et al. (8) |
|---|---|---|---|---|
| Age at first admission | 3-month | 7-month | 2-month | 2-month |
| Age at diagnosis of IPEX | 5-month | 42-month | 5-year | 9-year |
| Consanguinity | - | - | N/A | N/A |
| Family history of the findings | + | + | + | + |
| Eczematous skin rash | + | + | + | + |
| Hepatosplenomegaly | - | + | N/A | + |
| Reccurrent pneumonia | + | + | - | N/A |
| Chronic diarrhea | - | + | - | + |
| Hemoglobin (g/dL) | 7.1 | 9.9/11.4 | 7.8 | N/A |
| White blood cell count ($mm^3$) | 8700 | 13100/7300 | N/A | N/A |
| Eosinophilia (%) | 8.4 | 3.7/10.3 | N/A | N/A |
| Absolute neutrophil count ($mm^3$) | 3300 | 5700/3700 | N/A | N/A |
| Absolute lymphocyte count ($mm^3$) | 3700 | 5800/2700 | N/A | N/A |
| Direct Coombs test | + | - /NA | N/A | N/A |
| Serum albumin (g/dL) | 4.0 | 2.3 | N/A | N/A |
| Serum glucose (mg/dL) | 400 | 91/82 | 373 | N/A |
| Immunoglobulin G (mg/dL) | 856 | 762/652 | N/A | N/A |
| Immunoglobulin M (mg/dL) | 102 | 113/101 | N/A | N/A |
| Immunoglobulin A (mg/dL) | 30.8 | 85.6/43.8 | N/A | N/A |
| Immunoglobulin E (IU/mL) | 1538 | 1368/1042 | 1141 | N/A |
| Diabetes-related antibodies | + | NA/NA | N/A | N/A |
| Diabetes mellitus | + | - | + | + |
| AHA | + | - | + | - |
| Renal disease | - | + | + | -* |
| Hypothyroidism | - | - | - | - |
| Thrombocytopenia | - | - | - | + |
| Other | - | - | - | Arthritis |
| FOXP3 mutation c.748_750delAAG, p.250K.del | + | + | + | + |

*Reported progressive renal insufficiency secondary to long-term therapy with nephrotoxic drugs.
N/A: not available, AHA: autoimmune hemolytic anemia, IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked

in mind in a male patients who is diagnosed with diabetes in infancy, even though they have no other features of IPEX syndrome initially.

It has been suggested that up to one-third of IPEX patients have renal disease (2). Autoimmunity and long-term usage of nephrotoxic drugs are the main causes of renal disease in these patients. Renal involvement in IPEX syndrome can manifest as tubulointerstitial damage or glomerulopathy presenting as nephrotic syndrome (2,10,11). The mutation detected in the present family has been previously reported in a five-year old boy with IPEX syndrome with minimal change disease (7). Importantly, case 2 presented with a different clinical course from case 1, including mesangial proliferative glomerulonephritis, beside dermatitis, enteropathy, and hyperimmunoglobulin E, and he died at 4 years of age because of severe infection. The genotype and phenotype relationship was evaluated in a large IPEX cohort, but no unequivocal correlation was found (5). The findings in these presented siblings are consistent with a lack of genotype-phenotype correlation in IPEX patients, especially in the first years of the disease. Case 1 had no clinical and laboratory findings of renal disease before and after HSCT. However, Park et al. (11) reviewed the possible genotype-phenotype correlation and suggested that enteropathic presentations, eczema, AHA and food allergy were associated with better survival, while thrombocytopenia, septic shock and mutations affecting the repressor domain, intron 7 or poly A sequence were associated with increased risk of death. As the number of reported cases increases, genotype-phenotype correlations may become clearer and its importance in predicting the disease course will become better in the future.

The heterogeneous clinical features of patients with IPEX syndrome can cause difficulties in early diagnosis. The reason for clinical diversity, even in patients with the same mutation in IPEX syndrome, is controversial. Environmental factors have been suggested as a cause of this diversity but we suggest this could not be the case in our patients since they shared similar environmental factors (12). It is of interest whether clinical diversity in IPEX syndrome could be related to the disease onset since the patients were not followed for many years and the actual natural course of the disease and progression is unknown. Furthermore, management of IPEX syndrome may have an important role on the natural course of the disease. That is to say, renal disease and/or enteritis may have developed later in case 1 if the HSCT had not been performed, and case 2 might have developed type 1 diabetes mellitus and/or AHA if he hadn't died. Therefore, we wished to highlight the initial findings in terms of early diagnosis and treatment. The absence of the classic clinical triad of the disease may have led to a delay in diagnosis of IPEX syndrome in our older patient who did not have endocrinopathy. It is also recommended that molecular analysis should be considered to avoid delayed diagnosis.

In the family history of case 1, the clinical picture of his older brother helped to make an early diagnosis of IPEX syndrome, although case 2 also did not have the classic clinical triad.

## Conclusion

In this context, we would like to underline that family history should be taken in detail and carefully to avoid misdiagnosis regarding IPEX syndrome. We suggest that IPEX should be suspected in a male infant, not only in those with the classical triad, but also in those who have only one main disorder related to IPEX syndrome, such as infantile diabetes.

### Ethics

**Informed Consent:** Verbal and written parental informed consents were obtained for both siblings.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Gülay Karagüzel, Recep Polat, Mehtap H. Abul, Alper Han Cebi, Fazıl Orhan, Concept: Gülay Karagüzel, Fazıl Orhan, Design: Gülay Karagüzel, Data Collection or Processing: Gülay Karagüzel, Recep Polat, Mehtap H. Abul, Analysis or Interpretation: Gülay Karagüzel, Alper Han Cebi, Fazıl Orhan, Literature Search: Gülay Karagüzel, Recep Polat, Mehtap H. Abul, Alper Han Cebi, Fazıl Orhan, Writing: Gülay Karagüzel, Fazıl Orhan.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Dhuhan BK, Piccirillo CA. The immunological and genetic basis of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Curr Opin Allergy Clin Immunol 2015;156:525-532.
2. Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann NY Acad Sci 2018;1417:5-22.
3. Barzaghi E, Passerini L, Bacchetta R. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; a paradigm of immunodeficiency with autoimmunity. Front Immunol 2012;3:1-25.
4. Duclaux-Loras R, Charbit-Henrion F, Neven B, Nowak J, Collardeau-Frachon S, Malcus C, Ray PF, Moshous D, Beltrand J, Goulet O, Cerf-Bensussan N, Lachaux A, Rieux-Laucat F, Ruemmele FM. Clinical heterogeneity of immune dysregulation, polyendocrinopathy,

enteropathy, X-linked syndrome: A French multicenter retrospective study. Clin Transl Gastroenterol 2018;9:201-211.

5. Gambineri E, Mannurita SC, Hagin D, Vignoli M, Anover-Sombke S, DeBoer S, Segundo GRS, Allenspach EJ, Favre C. Ochs HD, Torgerson TR. Clinical, immunological and molecular heterogeneity of 173 patients with the phenotype of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Front Immunol 2018;9:2411-2429.
6. Blanco Quiro A, Arranz Sanz E, Bernardo Ordiz D, Garrote Adrados JA. From autoimmune enteropathy to the IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) syndrome. Allergol Immunopathol (Madr) 2009;37:208-215.
7. Hashimura Y, Nozu K, Kanegane H, Miyawaki T, Hayakawa A, Yoshikawa N, Nakaniski K, Takemoto M, Lijima K, Matsuo M. Minimal change nephrotic syndrome associated with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Pediatr Nephrol 2009;24:1181-1186.
8. Wildin RS, Smyk-Pearson S, Filipvich AH. Clinical and molecular feature of the immune dysregulation, polyendocrinopathy, enteropathy, x-linked (IPEX) syndrome. J Med Genet 2002;39:537-545.
9. Hwang JL, Park SY, Ye H, Sanyoura M, Pastore AN, Carmody D, Del Gaudio D, Wilson JF, Hanis JL, Liu X, Atzmon G, Glaser B, Philipson LH, Greeley SAW, T2D-Genes Consortium. FOXP3 mutations causing early-onset insulin-requering diabetes but without other features of IPEX syndrome: FOXP3 mutations with IPEX-like diabetes. Pediatr Diabetes 2018;19:388-392.
10. Bae KW, Kim BE, Choi J, Lee JH, Park YS, Kim GH, Yoo HW, Seo JJ. A novel mutation and unusual clinical features in patient with immune dysregulation, polyendocrinopathy, enteropathy, x-linked (IPEX) syndrome. Eur J Pediatr 2011;170:1611-1615.
11. Park JH, Lee KH, Jeon B, Ochs HD, Lee JS, Gee HY, Seo S, Geum D, Piccirillo CA, Eisenhut M, van der Vliet HJ, Lee JM, Kronbichler A, Ko Y, Shin JI. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: A systematic review. Autoimmun Rev 2020;19:102526.
12. Smith E, Greeley SAW, Ye H, Torgerson TR, Dimmitt R, Atkinson P, Philips J, Goldman F. Exremely early onset IPEX syndrome caused by a novel small exonic deletion in FOXP3. J Pediatr Gastroenterol Nutr 2016;63:119-120.

DOI: 10.4274/jcrpe.galenos.2022.2022-3-22

J Clin Res Pediatr Endocrinol 2022;14(3):366-367

# Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic

**Rujittika Mungmunpuntipantip[1], Viroj Wiwanitkit[2]**

*[1]Private Academic Consultant, Bangkok, Thailand*
*[2]Dr DY Patil University, Pune, India*

**Keywords:** Children, diabetes, COVID-19

**Dear Editor,**

We would like to share ideas on the publication "A Long-Term Comparison of Presenting Characteristics of Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic" (1). Kaya et al. (1) noted that increased frequency and severity of diabetic ketoacidosis (DKA) was observed in children with newly diagnosed with type 1 diabetes (T1DM) during the pandemic, and the results justify concerns about the diagnosis of other diseases during the pandemic. Kaya et al. (1) also stated that diabetes symptom recognition studies should be continued regularly to reach all parts of society during a pandemic. We agree that Coronavirus disease-2019 (COVID-19) can cause endocrine disruption and its correlation with diabetes is interesting. Severe acute respiratory syndrome-Coronavirus-2 may promote the beginning of T1DM and may hasten the emergence of DKA in juvenile diabetic patients, according to a report by Albuali and AlGhamdi (2), even in the absence of respiratory symptoms.

Kaya et al. (1) has found an increased frequency and severity of DKA in children with newly diagnosed T1DM in the pandemic period, and they are concerned about the diagnosis of other diseases during the pandemic. Although there might be limitation on controlling of confounding factors due to nature of a retrospective study based on records review, Kaya et al. (1) point to some interesting findings. Possible pathological associations may be due to an abnormal immune response/inflammation or increased blood viscosity due to COVID-19 (3,4,5). In brief, the pathogen might induce abnormal immunity that can directly attack islet cell or there might be hyperinflammatory process that can result in DKA (3,4). Additionally, the increased blood viscosity is a common observation in COVID-19 case (6). The hyperviscosity induced by COVID-19 might also be a triggering factor for DKA development (5). The current study has been able to show that there are no significant effects of immune/autoantibodies. The observation on no significant effects of immune/autoantibodies can be further interpreted in term of possible exact pathophysiology of DKA. Based on the data from the present study, the pathogenesis of DKA should be unlikely to be associated with abnormal immune response. This may mean that changes in blood viscosity after COVID-19 may contribute to the development of DKA.

### Ethics

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Writing: Rujittika Mungmunpuntipantip, Viroj Wiwanitkit.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Kaya G, Cimbek EA, Yeşilbaş O, Bostan YE, Karagüzel G. A Long-Term Comparison of Presenting Characteristics of Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic. J Clin Res Pediatr Endocrinol 2022;14:267-274.

**Address for Correspondence:** Rujittika Mungmunpuntipantip MD, Private Academic Consultant, Bangkok, Thailand
**E-mail:** rujittika@gmail.com **ORCID:** orcid.org/0000-0003-0078-7897

**Conflict of interest:** None declared
**Received:** 24.03.2022
**Accepted:** 10.05.2022

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

2. Albuali WH, AlGhamdi NA. Diabetic ketoacidosis precipitated by atypical coronavirus disease in a newly diagnosed diabetic girl. J Taibah Univ Med Sci 2021;16:628-631.

3. Eskandarani RM, Sawan S. Diabetic Ketoacidosis on Hospitalization with COVID-19 in a Previously Nondiabetic Patient: A Review of Pathophysiology. Clin Med Insights Endocrinol Diabetes 2020;13:1179551420984125.

4. Palermo NE, Sadhu AR, McDonnell ME. Diabetic Ketoacidosis in COVID-19: Unique Concerns and Considerations. J Clin Endocrinol Metab 2020;105:dgaa360.

5. Mungmunpuntipantip R, Wiwanitkit V. Diabetic ketoacidosis, COVID-19 and blood viscosity. J Taibah Univ Med Sci 2022;17:343-344. Epub 2021 Nov 11

6. Joob B, Wiwanitkit V. Blood viscosity of COVID-19 patient: a preliminary report. Am J Blood Res 2021;11:93-95.

DOI: 10.4274/jcrpe.galenos.2022.2022-3-22-reply
J Clin Res Pediatr Endocrinol 2022;14(3):368-369

# In response to: "Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic"

**Emine Ayça Cimbek[1], Gülay Kaya[2], Osman Yeşilbaş[3], Gülay Karagüzel[1]**

*[1]Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Turkey*
*[2]Karadeniz Technical University Faculty of Medicine, Department of Pediatrics, Trabzon, Turkey*
*[3]Hisar Intercontinental Hospital, Pediatric Intensive Care Unit, İstanbul, Turkey*

**Keywords:** COVID-19, diabetic ketoacidosis, type 1 diabetes

**Dear Editor,**

We would like to thank Mungmunpuntipantip and Wiwanitkit (1) for their interest in our study and for sharing their ideas. The authors have provided an insightful perspective on an abnormal immune response/inflammation or increased blood viscosity as a possible explanation for the association between type 1 diabetes (T1D)/diabetic ketoacidosis (DKA) and Coronavirus disease-2019 (COVID-19) by the relevant literature (2,3).

Several authors have suggested that COVID-19 can amplify an individual's risk of diabetes, primarily type 2 diabetes, months after the infection (4). People with a high body-mass index, a risk factor for type 2 diabetes already, had more than double the probability of developing diabetes after the infection. The risk of developing diabetes was also correlated with the severity of COVID-19. However, evidence on a link between COVID-19 and newly diagnosed T1D remains inconsistent. A recent study by van der Heide et al. (5), demonstrating the limits of pancreatic Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection, challenged the proposition that targeting of beta cells by SARS-CoV-2 precipitates new-onset diabetes. Salmi et al. (6) showed that the increased rate of severe DKA at diagnosis during the pandemic was not a consequence of COVID-19 in children. Instead, similar to us, they suggested that it might be related to delays in diagnosis following changes in parental attitudes and access to healthcare. Additionally, we would like to emphasize that although we've found an increased frequency and severity of DKA in children with newly diagnosed T1D in the pandemic period compared to the pre-pandemic period, PCR tests were administered to only six patients with a history of contact, revealing no COVID-19-positive case in our study (7). Thus, we think that a definite interpretation of the hyperviscosity or an immune response induced by COVID-19 as a triggering factor for DKA development could not be made based solely on our findings.

In summary, our study has investigated the presenting characteristics of newly diagnosed T1D patients during the pandemic and compared them with the pre-pandemic period. Our findings justify the concerns of delays in T1D diagnosis, among other diseases during the pandemic period, probably due to hesitations in referring to hospitals. Furthermore, strategies and guidance should be provided to empower clinicians and patients to avoid DKA when possible. Finally, further studies are warranted to investigate the possible association of restricted pancreatic damage, immunologic alterations/inflammation, or increased blood viscosity with T1D/DKA and COVID-19, as suggested by Mungmunpuntipantip and Wiwanitkit (1).

## Ethics

**Peer-review:** Internally peer-reviewed.

**Address for Correspondence:** Gülay Karagüzel MD, Karadeniz Technical University Faculty of Medicine, Department of Pediatric Endocrinology, Trabzon, Türkiye
**Phone:** +90 542 425 69 56 **E-mail:** gulkarag@hotmail.com, gulaykg@yahoo.com
**ORCID:** orcid.org/0000-0003-4116-5365

**Conflict of interest:** None declared
**Received:** 16.08.2022
**Accepted:** 17.08.2022

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The Journal of Clinical Research in Pediatric Endocrinology published by Galenos Publishing House.

### Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: All authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Mungmunpuntipantip R, Wiwanitkit V. Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic. J Clin Res Pediatr Endocrinol 2022;14:366-367. Epub 2022 May 31
2. Eskandarani RM, Sawan S. Diabetic Ketoacidosis on Hospitalization with COVID-19 in a Previously Nondiabetic Patient: A Review of Pathophysiology. Clin Med Insights Endocrinol Diabetes 2020;13:1179551420984125.
3. Mungmunpuntipantip R, Wiwanitkit V. Diabetic ketoacidosis, COVID-19 and blood viscosity. J Taibah Univ Med Sci 2022;17:343-344. Epub 2021 Nov 11
4. Xie Y, Al-Aly Z. Risks and burdens of incident diabetes in long COVID: a cohort study. Lancet Diabetes Endocrinol 2022;10:311-321. Epub 2022 Mar 21
5. van der Heide V, Jangra S, Cohen P, Rathnasinghe R, Aslam S, Aydillo T, Geanon D, Handler D, Kelley G, Lee B, Rahman A, Dawson T, Qi J, D'Souza D, Kim-Schulze S, Panzer JK, Caicedo A, Kusmartseva I, Posgai AL, Atkinson MA, Albrecht RA, García-Sastre A, Rosenberg BR, Schotsaert M, Homann D. Limited extent and consequences of pancreatic SARS-CoV-2 infection. Cell Rep 2022;38:110508. Epub 2022 Feb 21
6. Salmi H, Heinonen S, Hästbacka J, Lääperi M, Rautiainen P, Miettinen PJ, Vapalahti O, Hepojoki J, Knip M. New-onset type 1 diabetes in Finnish children during the COVID-19 pandemic. Arch Dis Child 2022;107:180-185. Epub 2021 May 27
7. Kaya G, Cimbek EA, Yeşilbaş O, Bostan YE, Karagüzel G. A Long-Term Comparison of Presenting Characteristics of Children with Newly Diagnosed Type 1 Diabetes Before and During the COVID-19 Pandemic. J Clin Res Pediatr Endocrinol 2022;14:267-274.