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Unique coexistence of *SHOX* and *PTHLH* gene mutations in a 12-year-old boy with syndromic short stature. Case report with literature review

Unikatowe współistnienie mutacji genów *SHOX* i *PTHLH* u 12-letniego chłopca z syndromicznym niskim wzrostem. Opis przypadku z przeglądem literatury

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Abstract

Despite the availability of advanced genetic testing that enables accurate and reliable genotype assessment, clinicians continue to face diagnostic problems, especially in patients with potentially pathogenic mutations in different genes. *SHOX* gene mutations are the most common cause of proportional monogenic short stature, whereas the *PTHLH* gene encodes a parathormone-related protein that plays a crucial role in the regulation of cell growth, calcium ion transport, and bone development. The authors present the case of a 12-year-old boy with a short stature and a mosaic of developmental malformations; nephrocalcinosis; and calcium and magnesium metabolism disorders; with a unique coexistence of mutations in the *SHOX* and *PTHLH* genes. The presented case extends the clinical spectrum associated with these rare mutations and shows the usefulness of whole exome sequencing in diagnosing patients with atypical phenotypes.

Keywords: mutation, short stature, hypercalciuria, nephrocalcinosis, hypermagnesuria

Streszczenie

Pomimo dostępności zaawansowanych testów genetycznych, umożliwiających dokładną i wiarygodną ocenę genotypu, klinicyści wciąż mierzą się z problemami diagnostycznymi, zwłaszcza u pacjentów, u których współistnieją potencjalnie patogenne mutacje różnych genów. Mutacje genu *SHOX* są najczęstszą przyczyną monogenowej proporcjonalnej niskorosłości, natomiast gen *PTHLH* koduje białko związane z parathormonem, które odgrywa kluczową rolę w regulacji wzrostu komórek, transportu jonów wapnia i rozwoju kości. Autorzy przedstawiają przypadek 12-letniego niskorosłego chłopca z mozaiką wad rozwojowych, nefrokalcynozą, zaburzeniami metabolizmu wapnia i magnezu oraz unikalnym współwystępowaniem mutacji genów *SHOX* i *PTHLH*. Prezentowany przypadek rozszerza spektrum kliniczne związane z tymi rzadkimi mutacjami oraz wskazuje na przydatność sekwencjonowania całego eksomu w wyjaśnianiu nietypowych fenotypów.

Słowa kluczowe: mutacja, niskorosłość, hiperkalciuria, nefrokalcynoza, hipermagnezuria

INTRODUCTION

Human embryogenesis is a complex process of the coordinated development of organs and tissues. It underlies strict molecular control involving the expression of specific genes and transcription factors. A failure of these mechanisms may lead to different phenotypic alterations. New molecular technologies, such as whole exome sequencing (WES), improve the quality of genotype assessment[1]. The results support clinical diagnosis, reveal genotype-phenotype correlations, enable genetic counselling, and provide new insights into the aetiopathogenesis of developmental and metabolic abnormalities. It is estimated that nowadays up to 25–40% of children with idiopathic short stature may be genetically diagnosed using these technologies[2].

In this report, we present the case of a 12-year-old boy with a short stature and a mosaic of developmental defects due to a pathogenic variant in the *SHOX* gene and a variant of unknown significance in the *PTHLH* gene, detected by WES when the boy was nine years old.

CASE PRESENTATION

The boy, the 5th child of non-consanguineous Caucasian parents (from the 6th pregnancy of a 40-year-old mother), was born at term after a pregnancy complicated by intrauterine growth retardation (IUGR). His physical parameters at birth were <3rd percentile (weight 2,540 g; length 47 cm; head and chest circumferences 30 and 27 cm, respectively). The Apgar scores were 8, 7, and 9 at 1, 3, and 5 minutes of life, respectively. Postnatally, he presented with disturbed breathing and required moderate oxygen supplementation. The initially suspected choanal atresia was excluded but labiomandibular hypotonia with impaired coordination of sucking, breathing, and swallowing was subsequently diagnosed. The boy required tube feeding support during the neonatal period. A hemodynamically significant complex congenital heart defect (CHD), comprising ventricular (VSD) and atrial (ASD II) septal defects, was revealed. It was associated with ventricular arrhythmias which were treated with propranolol and spironolactone. In addition, at the age of one month, bilaterally increased echogenicity of renal pyramids suggestive of nephrocalcinosis (NC) was found. Initial aetiological evaluation, including detailed blood biochemistry, screening for metabolic diseases, cystic fibrosis, TORCH, as well as karyotype analysis, and then FISH technique for 22q11.2 and 7q11.23 regions to confirm or exclude suspected DiGeorge and Williams syndromes, was negative. During the infantile period, the boy had recurrent urinary tract infections (UTIs), and bladder catheterisation problems were observed. Although neither widening of the pelvicalyceal system nor bladder abnormalities were found on ultrasound, voiding cystography at the age of 11 months was performed. The result was suggestive of posterior urethral valve (PUV) disorder. Urodynamic studies showed detrusor overactivity with sphincter–detrusor discoordination and voiding supported by increased abdominal pressure. At the age of 19 months, PUV was definitely confirmed by cystourethroscopy and then surgically incised. Therapy with an alpha-blocker (doxazosin) at a daily dose of 0.25 mg was introduced.

At the age of 13 months, propranolol treatment was discontinued. Echocardiography showed spontaneous occlusion of VSD, leaving only a small subaortic aneurysm, simultaneously "punctual" foramen ovale remained with no hemodynamic significance.

In the neurological follow-up, mild hypotonia, microcephaly (head circumference <3rd percentile), and minor psychomotor retardation were observed. Because of persistent NC, citrate supplementation was introduced (potassium citrate in a daily dose of 170 mg) seven months later.

At the age of six and a half years, hypercalciuria (HC) of 7.55 mg/kg/24 h (normal <4 mg/kg/24 h) was diagnosed for the first time in the local hospital. However, the treatment was not modified.

Six months later, the differential diagnosis of growth failure indicated a growth hormone (GH) deficiency in post-sleep GH stimulation test and normal pituitary gland on magnetic resonance imaging. Consequently, recombinant human growth hormone (rHGH) therapy was introduced.

At the age of eight, the boy was referred to our department for metabolic re-evaluation of NC. He presented with a proportional short stature (104 cm, <3rd percentile) and facial dysmorphia with a wide forehead, bulbous nose, relatively large auricles and earlobes, high-arched palate, crowding of teeth, malocclusion, and pectus excavatum (Fig. 1).

Laboratory tests showed HC (5.62 mg/kg/24 h), while the urinary excretion of oxalates (0.5 mmol/1.73 m^2/24 h; normal <0.5 mmol/1.73 m^2/24 h) and citrates (1.62 mmol/1.73 m^2/24 h; normal >0.94 mmol/1.73 m^2/24 h), as well as the fractional urinary excretion of phosphates, were acceptable. The boy's kidney function (estimated glomerular filtration rate, eGFR – 119 mL/min/1.73 m^2) and serum biochemistry including electrolytes, blood gases, parathyroid hormone, and metabolites of vitamin D_3 were normal. Densitometry showed decreased total body bone mineral density (TBBMD) with Z-score: –3.4, <3rd percentile. Ultrasound examination confirmed grade 1 NC according to Hoyer's classification[3] (Fig. 2).

To manage HC, treatment with hydrochlorothiazide (HT) at a daily dose of 0.5 mg/kg was started and effectively normalised urinary calcium excretion (<4 mg/kg/24 h) within the next months.

At the age of nine years, borderline magnesaemia (0.7 mmol/L) with slightly increased fractional urinary magnesium excretion (4.95%) was revealed, and magnesium citrate at a daily dose of 14 mg/kg of Mg^{2+} was started. In the follow-up, magnesium supplementation had to be increased to maintain a normal serum magnesium level.

As a syndromic association was suspected, WES along with the analysis of actionable genes according to the American

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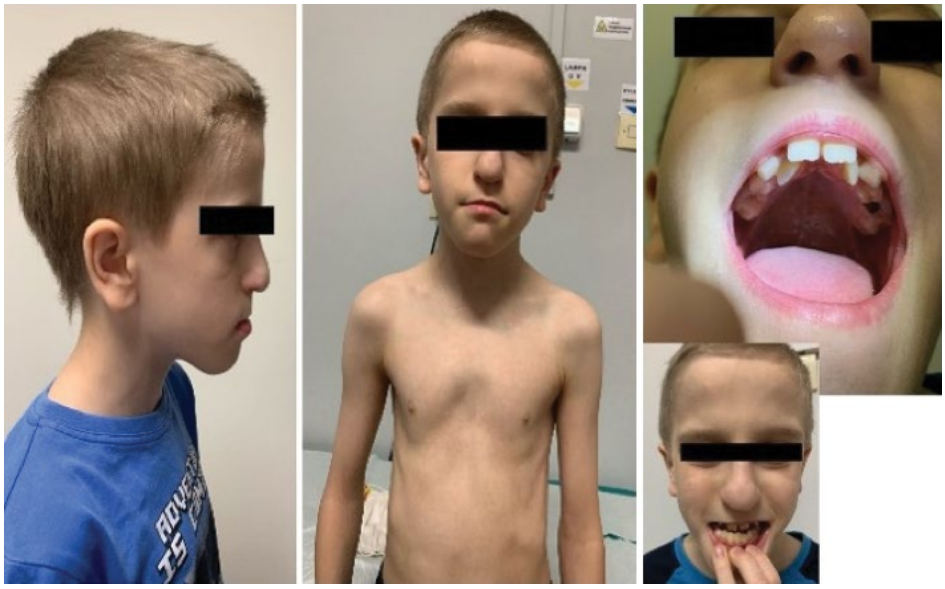

Fig. 1. Photographs of the presented patient. Note dysmorphic facial features including wide forehead, bulbous nose, relatively large auricles and earlobes, high-arched palate, crowding of teeth, and malocclusion as well as pectus excavatum

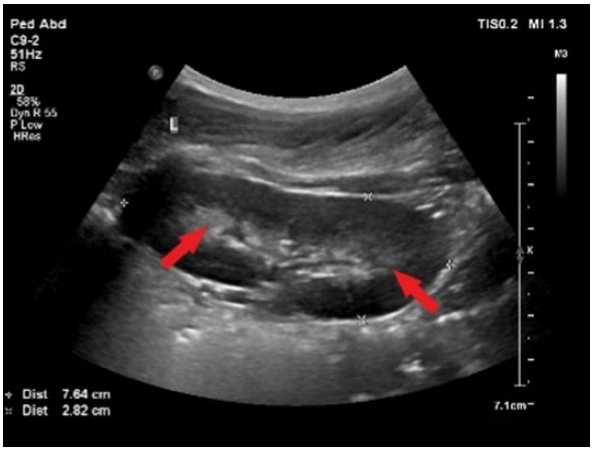

Fig. 2. Ultrasound of the patient's left kidney. Note increased echogenicity of the renal pyramids – arrows (Department of Paediatric Radiology, Medical University of Lublin)

College of Medical Genetics (ACMG) was performed when the boy was nine years old. It revealed heterozygous variants of two genes, the earlier reported pathogenic variant p.Lys29Thr in the *SHOX* gene (X-chromosome) and the novel variant (variant of unknown significance) p.Glu28Lys in the *PTHLH* gene (chromosome 12). Accordingly, the former was found in the patient's mother and the latter in his father. That was not surprising, as both parents are short-statured (father 165 cm, mother 149 cm). Moreover, the mother was born with congenital clubfoot. An X-ray of the patient's right hand performed at that time revealed brachymesophalangia type A4 of the 2nd and 5th fingers, and slight shortening of the 5th metacarpal bone (Fig. 3).

Follow-up densitometry performed at the age of 11.5 years showed normalisation of TBBMD (Z-score: 0.3; 37th percentile). Currently, at the age of 12, the boy is still short-statured despite rHGH therapy. His height (135.5 cm), weight

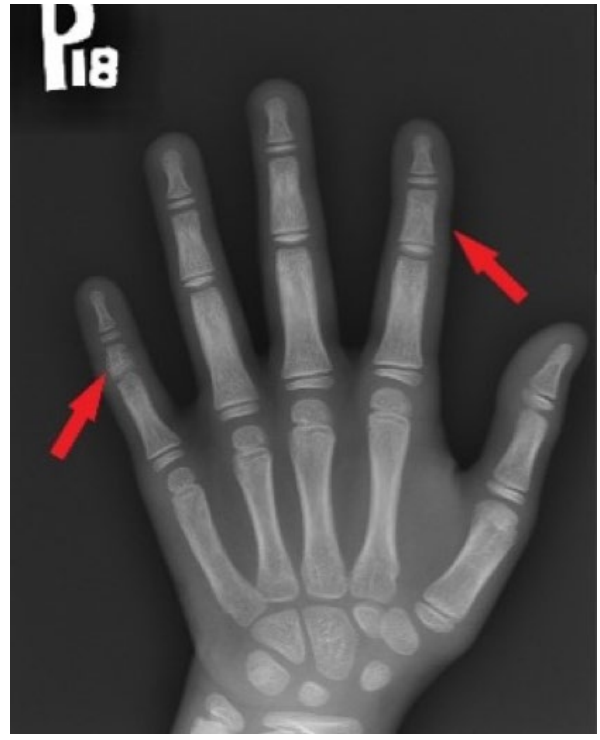

Fig. 3. X-ray of the patient's right hand at the age of 10. Note shortened 5th metacarpal bone and middle phalanges of the 2nd and 5th fingers – arrows (Department of Paediatric Radiology, Medical University of Lublin)

(24 kg), and chest circumference (56 cm) are <3rd percentile, while his head circumference is 52.5 cm (3rd–10th percentile). The boy's mental development is appropriate for his age. To address HC (4.5–6.4 mg/kg/24 h), the daily dose of HT was increased to 0.78 mg/kg. Simultaneously increasing magnesuria (5.87–9.4 mg/kg/24 h) requires supplementation of magnesium citrate at a dose of 16.5 mg/kg/24 h

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of Mg^{2+} to maintain normal magnesaemia (0.72 mmol/L). The levels of 25-hydroxyvitamin D (36.6 ng/mL), parathyroid hormone (17.7 pg/mL), calcaemia (2.54 mmoL/L), and phosphataemia (1.62 mmol/L), as well as kidney function (eGFR 152 mL/min/1.73 m^2), are normal. NC is not progressive on renal ultrasound. The patient is free of UTIs but still requires doxazosin treatment (2 mg daily) due to the symptoms of valve bladder syndrome. At present, no significant abnormalities are observed in echocardiography. The interatrial septum is continuous, and a tiny aneurysm after spontaneous closure of the perimembranous VSD is irrelevant.

DISCUSSION

Longitudinal growth is a complex process influenced by a variety of environmental and intrinsic factors. In recent decades, wide genomic analyses have provided new insights into the genetic regulation of chondrogenesis, and currently, next-generation sequencing serves as a useful diagnostic tool, especially in patients with short stature of unknown origin. It is obvious that growth is regulated by hundreds of genes, which are involved in the development and function of the growth plate. Specifically, they are responsible for hormonal regulation, paracrine signalling, cartilage extracellular matrix function, and fundamental cellular processes[4].

In the presented patient, a wide spectrum of disorders was associated with IUGR, and later with short stature, which suggested a genetic basis. The patient's phenotype with CHD, IUGR, nephrocalcinosis, relatively high calcaemia, HC, hypotonia, breathing disorders in the neonatal period, growth retardation, and characteristic dysmorphic features with a broad forehead and high-arched palate initially suggested the diagnosis of Williams or DiGeorge syndromes[5] but microdeletions in the 7q11.23 and 22q11.2 regions were ruled out at the age of one year. However, WES performed when the boy was nine years old revealed a pathogenic variant in the *TBX1* gene, which could confirm the initial suspicion of DiGeorge syndrome. Due to the low coverage and accuracy of the examined region, genetic sequencing using the Sanger technique was carried out. It excluded the pathogenic variant in the *TBX1* gene identified in WES, which was considered an artefact due to limitations of the technology. Surprisingly, though, a pathogenic variant in the *SHOX* gene and a variant of unknown significance (VUS) in the *PTHLH* gene were found. The *SHOX* gene is located on both the X and Y chromosomes in an area called the pseudoautosomal region 1 (PAR1), and it encodes the SHOX protein, which is a transcription factor in the early embryonic phase. It is essential for the differentiation and maturation of chondrocytes as well as the growth of long bones. Mutations of the gene usually disturb foetal development, including IUGR, and cause post-natal growth disorders. The latter may be responsible for a broad phenotypic spectrum including proportional dwarfism with microcephaly,

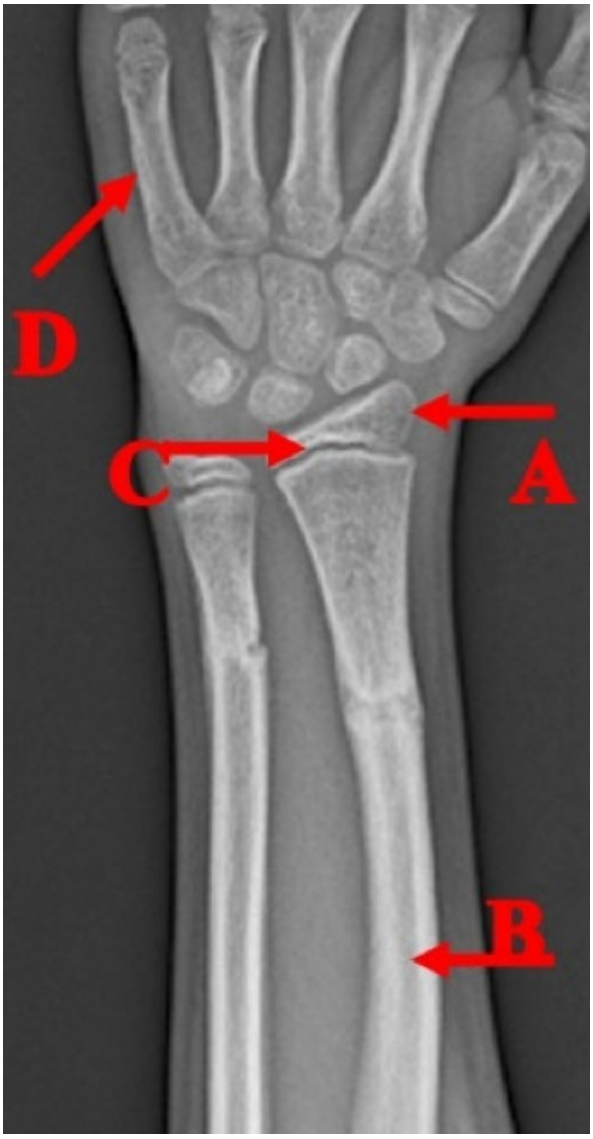

Fig. 4. X-ray of the patient's left hand, obtained at the age of 11.5 due to forearm fracture. Note slight triangularisation of the distal radial epiphysis (arrow A), slightly enlarged diaphysis of radius and marked bowing of radius (arrow B), convexity of the distal radial metaphysis (arrow C), and short 5th metacarpal (arrow D). X-ray provided by the patient's mother

skeletal dysplasia, facial dysmorphia, and other extra-skeletal abnormalities. A *SHOX* gene defect is the most common cause of monogenic short stature[6]. Homozygous mutations may lead to Langer mesomelic dysplasia which is characterised by severe short stature with extreme shortening of the limbs and so-called Madelung's deformity of the wrist (bowing of the radial shaft with increased interosseous space and dorsal subluxation of the distal radioulnar joint). Heterozygous mutations may be responsible for milder skeletal dysplasia, Léri–Weill dyschondrosteosis (LWD), but they are also found in non-syndromic dwarfism. It is believed that *SHOX* haploidy is partially responsible for short stature and skeletal abnormalities in Turner syndrome[7]. The presented patient was born with IUGR,

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and post-natal catch-up growth was not observed. Being heterozygous, the patient has milder skeletal symptoms revealed on X-rays, like shortened 5th metacarpal bones, slight triangularisation of the distal radial epiphysis, mildly enlarged diaphysis of radius, marked bowing of radius, and convexity of the distal radial metaphysis (Fig. 4). Other radiological features of *SHOX* gene deficiency, including lucency of the ulnar border of the distal radius, short 4th metacarpal, and pyramidalisation of the carpal row, were not found in the presented patient[6,8].

Recently, Sadler et al. reported that the *SHOX* gene and regulatory regions might be implicated in the pathogenesis of clubfoot[9]. Interestingly, LWD phenotype may also include extra-skeletal features such as high body mass index, metabolic syndrome, and type 2 diabetes mellitus (DM2)[10]. Therefore, heterozygosity of the known pathogenic p.Lys29Thr variant of the *SHOX* gene in the patient's mother could contribute to her short stature, DM2, and clubfoot. It might also be responsible for the short stature of other family members, like the grandmother and the mother's sister. Interestingly, the phenotypes of affected individuals may be highly variable, even within the same family[8].

The second variant (of unknown significance) was detected in the *PTHLH* gene which belongs to a group of genes that are responsible for the regulation of paracrine signalling. It is located on the short arm of chromosome 12, and it encodes a parathyroid hormone-related protein (PTHrP), a neuroendocrine peptide that plays a key role in cell growth regulation, bone development, and calcium ion transport. Specifically, it regulates the balance between chondrocyte proliferation and the onset of hypertrophic differentiation during endochondral bone development. Mutations of the *PTHLH* gene are responsible for most cases of humoral malignant hypercalcaemia and autosomal dominant brachydactyly type E2 (BDE2)[11,12]. The latter is characterised by variable shortening of the metacarpal and/or metatarsal bones, short stature, and mild craniofacial dysmorphia. Some pathogenic variants in the *PTHLH* gene may cause a specific pattern of short stature accompanied by other clinical features such as oligodontia, delayed tooth eruption, and variable middle or distal phalangeal anomalies. Delayed bone age and chest deformities have been also reported[11,13]. These phenotypic features seem to be concordant with brachymesophalangia II and V type A4, dental anomalies, and thoracic anomalies in our patient. As the p.Glu28.Lys variant in the *PTHLH g*ene has not been reported so far, its phenotypic significance must be confirmed by other observations. This variant was inherited from the patient's father who is of short stature without other anomalies.

Thus, the pathogenic variant in the *SHOX* gene and the variant detected in the *PTHLH* gene might contribute to the presented patient's short stature. Interestingly, five-year therapy with rHGH was unsatisfactory.

Kasprzyk et al., in their study including 57 prepubertal patients, proved that responsiveness to rHGH therapy in patients with Turner syndrome depended on the type of karyotype abnormalities causing the syndrome[14]. There are some reports showing beneficial effects of rHGH treatment in patients with *SHOX* haploinsufficiency, but its effectiveness seems to be related to the mutation type[7,15]. We hypothesise that the poor outcome of rHGH therapy in the presented patient may be the result of an additive effect of detected variants in both the *SHOX* and *PTHLH* genes.

Other disorders observed in our patient include nephrocalcinosis, HC, and hypermagnesuria. To the best of our knowledge, to date, they have not been reported in patients with pathogenic variants in the *SHOX* and *PTHLH* genes as well as PUV, hypotonia, and heart defects.

In the reported patient, HC was one of the first symptoms. PTH, vitamin D_3 metabolites, and phosphatemia were normal, while calcaemia tended to be in the upper normal range. Age-worsening hypermagnesuria resulting in hypomagnesemia occurred only at the age of nine and required increasing supplementation. Initially observed osteopenia could be a consequence of those disorders. Treatment, including HT and magnesium, resulted in the normalisation of calciuria, magnesaemia, and TBBMD, though careful monitoring and ongoing dose modifications are necessary.

CONCLUSIONS

The presented case is an interesting example of the unique coexistence of a pathogenic variant in the *SHOX* gene and a variant of unknown significance in the *PTHLH* gene in a 12-year-old boy with a short stature and a mosaic of developmental disorders. The case highlights the usefulness of WES diagnostics in explaining atypical phenotypes. Although the prognosis remains unclear, the authors indicate the importance of individualised and multidisciplinary care.

Statement of ethics

Written informed consent to publish the case with accompanying images was obtained from the patient's mother.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organisations which might negatively affect the content of this publication and/or claim authorship rights to this publication.

Author contributions

Original concept of study: ASD. Collection, recording and/or compilation of data: ASD. Analysis and interpretation of data: ASD, KW. Writing of manuscript: ASD. Critical review of manuscript: ASD, KW. Final approval of manuscript: ASD, KW.

References

1. Warr A, Robert C, Hume D et al.: Exome sequencing: current and future perspectives. G3 (Bethesda) 2015; 5: 1543–1550.
2. Murray PG, Clayton PE, Chernausek SD: A genetic approach to evaluation of short stature of undetermined cause. Lancet Diabetes Endocrinol 2018; 6: 564–574.
3. Hoyer PF: Nephrocalcinose. In: Hofmann V, Deeg KH, Hoyer PF (eds.): Ultraschalldiagnostik in Pädiatrie und Kinderchirurgie. Thieme, Stuttgart 1996: 372–374.
4. Mastromauro C, Chiarelli F: Novel insights into the genetic causes of short stature in children. touchREV Endocrinol 2022; 18: 49–57.
5. Stanley TL, Leong A, Pober BR: Growth, body composition, and endocrine issues in Williams syndrome. Curr Opin Endocrinol Diabetes Obes 2021; 28: 64–74.
6. Vannelli S, Baffico M, Buganza R et al.: SHOX deficiency in children with growth impairment: evaluation of known and new auxological and radiological indicators. Ital J Pediatr 2020; 46: 163.
7. Fukami M, Seki A, Ogata T: *SHOX* haploinsufficiency as a cause of syndromic and nonsyndromic short stature. Mol Syndromol 2016; 7: 3–11.
8. Gürsoy S, Hazan F, Aykut A et al.: Detection of *SHOX* gene variations in patients with skeletal abnormalities with or without short stature. J Clin Res Pediatr Endocrinol 2020; 12: 358–365.
9. Sadler B, Haller G, Antunes L et al.: Rare and *de novo* duplications containing *SHOX* in clubfoot. J Med Genet 2020; 57: 851–857.
10. Wang D, Pan X, Wang X: Metabolic syndrome coexists with adult Léri–Weill dyschondrosteosis: a case report. J Diabetes Investig 2021; 12: 446–449.
11. Jamsheer A, Sowińska-Seidler A, Olech EM et al.: Variable expressivity of the phenotype in two families with brachydactyly type E, craniofacial dysmorphism, short stature and delayed bone age caused by novel heterozygous mutations in the *PTHLH* gene. J Hum Genet 2016; 61: 457–461.
12. Wysolmerski JJ: Parathyroid hormone-related protein: an update. J Clin Endocrinol Metab 2012; 97: 2947–2956.
13. Tacke CE, Terheggen-Lagro SWJ, Boot AM et al.: Chondrodysplasia, enchondromas and a chest deformity causing severe pulmonary morbidity in a boy with a *PTHLH* duplication: a case report. Bone Rep 2021; 14: 101067.
14. Kasprzyk J, Włodarczyk M, Sobolewska-Włodarczyk A et al.: Karyotype abnormalities in the X chromosome predict response to the growth hormone therapy in Turner syndrome. J Clin Med 2021; 10: 5076.
15. Donze SH, Meijer CR, Kant SG et al.: The growth response to GH treatment is greater in patients with *SHOX* enhancer deletions compared to *SHOX* defects. Eur J Endocrinol 2015; 173: 611–621.

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