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Key words

Short stature, genomic variants, skeleton, sequencing

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Background: Congenital skeletal abnormalities are a heterogeneous group of diseases most commonly associated with small or disproportionate growth, cranial and facial dysmorphisms, delayed bone maturation, etc. Nonetheless, no detailed genotype-phenotype correlation in patients with specific genetic variants is readily available. Ergo, this study focuses on the analysis of patient phenotypes with candidate variants in genes involved in bone growth as detected by molecular genetic analysis.

Methods: In this study we used molecular genetic methods to analyse the *ACAN, COL2A1, FGFR3, IGFALS, IGF1, IGF1R, GHR, NPR2, STAT5B* and *SHOX* genes in 128 Czech children with suspected congenital skeletal abnormalities. Pathogenic variants and variants of unclear clinical significance were identified and we compared their frequency in this study cohort to the European non-Finnish

population. Furthermore, a prediction tool was utilised to determine their possible impact on the final protein. All clinical patient data was obtained during pre-test genetic counselling.

Results: Pathogenic variants were identified in the *FGFR3, GHR, COL2A1* and *SHOX* genes in a total of six patients. Furthermore, we identified 23 variants with unclear clinical significance and high allelic frequency in this cohort of patients with skeletal abnormalities. Five of them have not yet been reported in the scientific literature.

Conclusion: Congenital skeletal abnormalities may lead to a number of musculoskeletal, neurological, cardiovascular problems. Knowledge of specific pathogenic variants may help us in therapeutic procedures.

Key words

Short stature, genomic variants, skeleton, sequencing

Introduction

Congenital skeletal abnormalities are a group of disorders associated with abnormal bone formation caused by an intrinsic defect in the growth, development, and/or differentiation of specialised skeletal cells. Skeletal abnormalities are often genetic in nature. All in all, this group of diseases affects about one in 1,000 individuals globally. Studies of this disease are of high clinical importance (e.g. prenatal diagnosis, targeted therapy, prevention in second pregnancy) and provide information on the mechanism of the development and maintenance of the human skeleton (Guo L et al. 2023, Murrin EM et al. 2022, Savarirayan R et al. 2018). Congenital skeletal abnormalities may be difficult to diagnose due to the large number of various factors that need to be considered. They can be associated with facial and cranial defects, disproportionate stature or delayed bone maturation (Lemire EG, et al. 1998, Stolerman ES et al. 2019, Stattin EL et al. 2008, Cavallo F et al. 2021). Patient phenotypes may also include shortened or curved fingers, aberrant development of joints and spine, etc. (Lee MK et al. 2022, Litrenta J, et al. 2021, Blevins K et al. 2018). The disorder can also be manifested by an inconspicuous phenotype. The most common example of such a manifestation is short stature (Geister KA et al. 2015). The height of an individual is mainly determined by endochondral ossification chondrocytes included in the growth plate. Its coordination is based on chondrocytes and several humoral factors including growth hormone, parathyroid hormone, oestrogen, growth factors, cytokines and various signalling pathways (Tiffany AS et al. 2022, Knuth C et al. 2019). The highly complex regulatory signals involved in this process are genetically determined. Defects in any of the associated genes may lead to bone growth abnormalities (Ağırdil Y et al. 2020). It is very difficult to determine the specific causative agent of the disease due to the large number of genes involved in this process. The best current tool for genetic analysis is NGS (Next Generation Sequence). NGS enables the detection of genetic aberrations in many genes in dozens of patients in a short time (Matthijs et al. 2016). Despite the large testing capacity of NGS, it is more advantageous in clinical practise to narrow down the selection of candidate genes according to the specific phenotype of the patient or presumed heredity. There are panels containing a large number associated with skeletal aberrations, but their selection is very well controlled by clinical entities (e.g., *PTPN11* Noonan syndrome, *FGFR3* achondroplasia, etc.). However, for patients with an inconspicuous phenotype, such testing may be unnecessarily extensive and costly. Therefore, this study is focused on searching for new pathogenic variants associated with congenital skeletal abnormalities that manifest in an inconspicuous phenotype.

Material and methods

Patient cohort

The study included 128 children referred from endocrinology-specialised outpatient offices based on their clinical picture with suspected congenital bone aberrations (e.g. short stature, disproportionate stature, facial dysmorphism, unusual shape or length of fingers, etc.) between July 2021 and August 2022. No cause of the aberrant phenotype (e.g. growth hormone decline, foetal alcohol syndrome, etc.) had been detected in this cohort. Hence, they were referred for a genetic consultation at the Department of Medical Genetics at the Olomouc University Hospital. Written informed consent was obtained from the legal representatives of all study participants (**Appendix 1**). Study approval was obtained from the Institutional Review Board of the University Hospital and the Faculty of Medicine and Dentistry, Palacky University, Olomouc (Nr. 84/19). All procedures were conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice and valid legal regulations. The presented cohort included 74 girls and 54 boys of Czech nationality. Clinical records were available for 108 patients. The average age was 96.24 months (26-117 months) for girls and 96.76 months (25-213 months) for boys. Z-score for height was assigned based on the reference tables available at the website of the World Health Organization (WHO) and ranged from -3.94 to 0.96 (girls from -3.40 SDS to +0.96 SDS, boys from -3.94 SDS to +0.04 SDS). Paediatric endocrinologists ruled out a hormonal or metabolic disorder in these subjects. Chromosomal aberrations <10 Mb were excluded via conventional karyotyping and microarray performed at our department.

Molecular genetic analysis

Genes encoding components of the extracellular matrix of chondrocytes in cartilage (*ACAN, COL2A1*), specificities of the GH/IGF1 axis (*GHR, STAT5B, IGF1, IGFALS*) and signalling pathways in chondrocytes (*IGF1R, NPR2*) were selected for this study after discussion among genetic counsellors, endocrinologists, paediatricians and specialist in the laboratory field. Additionally, we selected genes for which pathogenic variants were also associated with mild or non-specific clinical pictures using the ClinVar and Online Mendelian Inheritance in Man (OMIM) databases and literature as well (Landrum MJ et al. 2018, Amberger JS et al. 2019, Jee YH et al. 2017, Burrage LC et al. 2013, Plachy L 2021, Plachy L 2023, Pesl M 2022, Klammt J 2018). Schema of its role in bone growth is shown in **figure 1**. Furthermore, we included the *SHOX* and *FGFR3* genes as their aberrations are known to cause special clinical phenotypes. DNA was isolated from peripheral blood by the salting-out method (Miller, 1988) or by automated isolation according to the manual of the manufacturer of the utilised commercial kit and instrument (Qiagen, ID of isolator 9002864, ID of kit 69516). Isolated samples went through library preparation according to the processing protocol (KAPA HyperPlus Kit, Roche Sequencing, **Appendix 2**). Targets were selected using custom primers (KAPA hyperchoice MAX 0.5 Mb T2, Roche, **Appendix 3**). The sequencing itself and raw data acquisition was performed with a MiSeq device (Illumina). Areas not covered are listed in **Table 1**. Sanger sequencing with specified primers was utilised to cover both alternative terminal exons of *SHOX* (exon 6) and to confirm pathogenic single nucleotide variants (SNVs) (Table 2). Templates were generated with PPP Master Mix (Top-Bio s.r.o., ID: P124). Following sequencing was prepared according manufactures protocols of BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™, ID: 4337455). SeqStudio™ Genetic Analyzer System with SmartStart (Applied Biosystems™, ID: A35644) was used for capillary electrophoresis and digitalization of signal. Example of results of pathogenic variant are shown in **Appendix 4**. Detected copy number variants (CNVs) were confirmed by multiplex PCR (Multiplex Ligation-dependent Probe Amplification, MLPA) with P018-SHOX probes (MRC Holland).

Data analysis

Cloud based tool BaseSpaceTM Sequence Hub (BSSH) (Illumina) using application FASTQ GenerationTM was automatically launched after sequencing for demultiplexing and generation of FASTQ files. Then data were aligned to reference genome hg19 and variants were called by NextGene software (SoftGenetics) resulting in .BAM and .vcf files. In the primary analysis, the signals acquired from the sequencer were converted into FASTQ files containing the sequences of individual reads combined with data quality information for each base. Only bases with a Phred score greater than 30 were chosen for further processing. Secondary analysis compared the results with reference human genome (GRCh37/hg19) and differences were sought (variant calling). Variants were called from raw data using the NEXTgene program. Exon 12 in *ACAN* (NM_001135.3) was excluded from the analysis. This region was not analysed since it included tandem repeats and the acquired results did not meet the minimum data quality requirements. The analysis focused on the variants in gene coding regions (exons), untranslated regions (UTRs) and near intronic regions (+/- 20 nt). Variants were classified according to ACMG criteria, prediction tools implemented in the Varsome tool and clinical-genetic correlation (Monaghan KG et al. 2013, Kopanos C et al. 2019). Variants of unclear clinical significance occurring in the non-Finnish general population with a frequency below 1% according to the gnomAD database were selected for further analysis (https://gnomad.broadinstitute.org/). Fisher's exact test was performed in these variants to detect variants occurring more frequently in this cohort than in the general population. These variants were additionally analysed using the Predct SNP2 prediction tool. The results of the stated tool were expressed as a ratio (damaging:benign effect of the variant) (Bendl J et al. 2016).

Results

In the cohort of 128 patients, pathogenic variants in the *FGFR3, GHR, COL2A1* and *SHOX* genes were detected in six patients (6/128, 4.7%). Five SNVs and one CNV were captured. Pathogenic SNVs were most often found in the *FGFR3* gene (n = 3), while the *GHR* and *COL2A1* genes only carried pathogenic SNVs in one patient. These pathogenic variants have not been detected in 'the Czech digital genome map project' yet (https://czechgenome.cz/). One pathogenic CNV–was detected in the *SHOX* gene (**Table 3**).

Variants in the *FGFR3* gene were detected in the protein kinase domain (c.1657G>A, c.2005C>G) and Ig-like C2-type 3 (c.943A>C) regions of the gene. The clinical picture of the carriers of these variants and their segregation in carrier families are presented here.

The c.943A>C variant was detected in a boy who acquired it *de-novo*. At the age of 13 years and 3 months, he was 156.8 cm tall (z-score -0.04). The endocrinologist reported asymmetric growth, facial dysmorphia, irregular tooth growth, delayed bone age. *Pubertas tarda* (penis = 4 cm, immersed in subcutaneous fat; testes in situ, approx. 3 ml), delayed development of speech and motor skills, impaired spatial vision and ventricular extrasystoles were also reported.

Variants of *FGFR3* protein kinase domain were detected in unrelated girls. A girl with the c.1657G>A variant was primarily referred for genetic testing because of short stature (Z-score -2.53). She inherited this variant from her father, who is 179 cm tall (**Figure 2**). The patient had a normal weight (22.7 kg) and head circumference (51.5 cm). Furthermore, she had a round face, gentle, without dysmorphic features, she had a smaller chin. Her figure was symmetrical and proportional. Her hands, nails, palmar grooves were unremarkable.

Another maternally inherited variant of c.2005C>G was detected in a girl with a conspicuous phenotype. This patient had a triangular face shape, high broad forehead, hypertelorism, wide nasal root, long upper body, wider chest, short fingers, especially the 3rd finger. Her height was borderline (Z-score -1.79). The weight (16.7 kg) and head circumference (51.0 cm) were adequate. Furthermore, bluish sclerae, short philtrum, narrow lips and flat feet were detected bilaterally (**Figure 3**).

The pathogenic variant of NM_000163.5: c.440-1G>A in *GHR* was found in a boy with borderline height (111 cm, Z-score -1.46) with slightly delayed bone age. At the age of 5 years and 5 months, he had a low weight of 16.7 kg (Z-score -2.62), but normal head circumference (52.0 cm). The boy had an oval face, broad nasal bridge, anti-Mongoloid slant of eye slits, low-set ears, atypical palmar grooves, bilateral flat feet and shorter toes. The boy inherited this variant from his mother, who is 160 cm tall. The boy's grandmother, who is 158 cm tall, did not undergo genetic testing (**Figure 4**).

The NM_001844.5:c.1636G>A variant in the *COL2A1* gene was detected in a girl with short stature (111 cm, Z-score -2.1). On the other hand, accelerated bone age was observed in this patient. The girl has no dysmorphic features or congenital developmental defects. Her figure is symmetrical. At the age of 7 years and 4 months, she has grade I obesity (26.3 kg, Z-score 2.2). The girl inherited this variant from her father, who is 160 cm tall. The father's mother, who is 160 cm tall, did not undergo relevant examinations (**Figure 5**).

A pathogenic CNV was found in the regulatory regions of the *SHOX* gene (NC_000023.11:g.(?_780700)_(835572_?)del) and its origin was not determined. The carrier was a girl who was examined at the age of 14. Her height was normal 160 cm (z-score -0.9). She had a proportional figure (with the exception of her arms), a mesocephalic head, an oval face, a higher forehead, without significant dysmorphic features. However, she had shorter arms with Madelung deformity after surgical correction. The origin of this variant was not determined (**Figure 6**).

Furthermore, 23 clinically unclear variants in *ACAN, COL2A1, FGFR3, IGFALS, IGF1R, GHR, NPR2 and SHOX* were detected in the studied cohort. These variants occurred significantly more commonly in the study group than in the general European non-Finnish population. Fourteen variants were identified in gene exons (8 missense and 6 synonymous), three in UTRs and six in introns (**Table 4**). The Predict SNP2 prediction tool detected seven variants that may have a damaging effect on the resulting protein, five variants were not identified and one reached a p-value < 0.05. The clinical features of the twelve candidate variants are summarised in **Table 5**. Detected VUS variants require further investigation via functional in vitro or in vivo studies to support their involvement in the short stature disorders. These investigations however were beyond the scope of this study.

Discussion

Fibroblast growth factor receptor 3 (*FGFR3*)

The FGFR3 receptor is one of four distinct membrane tyrosine kinases with the function of high-affinity receptors for a variety of fibroblast growth factors. Although this protein family plays a major role in various developmental processes, analyses have shown high levels of *FGFR3* expression in cartilaginous rudiments of a wide variety of bones (Nakajima A et al. 2003). In general, pathogenic variants of the *FGFR3* gene hold the receptor in an active state. This leads to premature differentiation of chondrocytes into mature osteoblastic cells, which is associated with increased apoptosis. (Chen J et al. 2017, Wilkie AO et al. 2005). As for the clinically described phenotypes interfering with bone development, the following may be listed: achondroplasia (ACH, OMIM: 100800); hypochondroplasia (HCH, OMIM: 146000); type I and II thanatophoric dysplasia (TDI, OMIM: 187600; TDII, OMIM: 187601); severe achondroplasia with developmental delay and acanthosis nigricans abbreviated as SADDAN (OMIM: 616482); camptodactyly with tall stature and hearing loss syndrome aka CATSHLS syndrome (OMIM: 610474); Crouzon syndrome with acanthosis nigricans (OMIM: 612247), lacrimo-auriculo-dento-digital syndrome 2 abbreviated LADD syndrome 2 (OMIM: 620192) and Muenke syndrome (OMIM: 602849). In addition to these, somatic pathogenic variants in *FGFR3* are associated with carcinogenesis. The clinical picture of the patient is unique for each variant and depends on its location. E.g., variants in IgIII are associated with HCH, presenting with a relatively mild phenotype, while SADDAN only develops with variants in tyrosine kinase domain 2 (TK2). Pathogenic variants of the *FGFR3* gene are also responsible for craniofacial aberrations (Harada D et al. 2009, Bellus GA et al. 1999, Heuertz S et al. 2006, Brodie SG et al. 1999, Li D et al. 2006, Bonaventure 2005, Nakajima 2003, Britto 2001).

In this study we presented three variants in the *FGFR3* gene that we identified as pathogenic. The c.943A>C variant was detected in a boy who acquired it *de-novo*. The boy does not suffer from growth restriction, but his physical disproportion is noticeable. The endocrinological examination reports delays in his bone age, motor and speech development. The patient also has irregular tooth growth. These features closely resemble LADD 2 syndrome with autosomal dominant inheritance. The detected variant is located in the extracellular part of the receptor in the IgIII region. Reported pathogenic variants in the same region of the receptor (e.g. p.Ser279Cys, p.Ser348Cys, p.Val342Phe, etc.) have been associated with the mild hypochondroplasia, achondroplasia/severe hypochondroplasia phenotype, disproportionate dwarfism with normal short limbs, mild brachydactylia and varying intelligence. The Z-score of carriers was less than -2.0 (Bengur 2019, Yao 2019, Song 2012, Heuertz 2006, Harada D et al. 2009). However, our patient does not fit these phenotypes. The prediction tool evaluated the variant as damaging to the resulting protein, the specific site is relatively deeply conserved (PhyloP100 = 7.19). Moreover, Ig variants are rare compared to TK variants (Wilkie AO et al. 2005). This study is the first to present the clinical phenotype of the c.943A>C variant.

This study detected two variants of FGFR3 in the tyrosine kinase domain. The c.2005C>G variant was detected in a girl with facial dysmorphic features, a disproportionate stature and a height 1.79 SDS below the mean. The girl inherited the variant from her mother who was 158 cm tall. This phenotype corresponds very well to HCH. This disease is characterised by autosomal dominant inheritance which was observed in our case as well. The adult height of the carrier of the pathogenic variant is between 125 and 160 cm. HCH may also present with shortened upper limbs, lumbar lordosis or mild facial hypoplasia, macrocephaly (Glasgow JF et al. 1978). However, these clinical features were not present in our patient. The c.2005C>G variant results in amino acid substitution (p.Arg669Gly), which has been identified as the cause of enhanced downstream signalling in bone marrow cells even at low levels of aberrant *FGFR3* expression (Patani H et al. 2016). Although the ClinVar database considers it a variant of unclear clinical significance, our study finds it clearly pathogenic based on the clinical picture of HCH, co-segregation with the phenotype in the family, low frequency in the population (f = 0.00000403) and the evaluation of the prediction tool as pathogenic for the resulting protein.

While the c.2005C>G variant corresponded very well to the HCH phenotype, the situation differed in the c.1657G>A variant in the *FGFR3* gene. This variant is also located in the TK domain and leads to amino acid substitution (p.Val553Met). However, the carrier of the variant failed to exhibit the typical HCH picture. Her figure was symmetrical, but her height was reduced by 2.53 SDS. In addition, HCH is inherited in an autosomal dominant manner (Ornitz 2017). However, this variant was inherited from her father, who was 178 cm tall, contrary to the expected final height (125-160 cm) in HCH. We have not been able to confirm the segregation of the phenotype with the variant in this case. On the other hand, the mother was only 158 cm tall. The mismatch of DNA samples of the parents was excluded in the laboratory by STR analysis. The occurrence of the variant in the population is an order of magnitude higher than that of the previous variant (f = 0.0000632), but the prediction tool considers this variant damaging for the resulting protein. The region of the variant is also highly conserved evolutionarily (PhyloP100 = 7.74). The variant also meets the ACMG criteria for probable pathogenic classification. On the other hand, the ClinVar database considers it unclear and, recently, benign without clinical correlation. Reduced penetrance and variable expressivity contribute to a wide spectrum of clinical findings. These phenomena have been described, e.g., in Muenke syndrome (Doherty ES et al. 2007). On the other hand, the penetrance of ACH is 100% (Daugherty A et al. 2017). Another possible explanation could be the presence of compound heterozygote. Autosomal recessive inheritance has already been described in CATSHL syndrome (Colvin JS et al. 1996). The second allele may be affected by altered methylation profile. However, our NGS method set up does not allow for the analysis of this factor. Differences in the level of CpG methylation between male and female germ cells may lead to sex-specific patterns (Crow JF. et al. 1997, Hall E et al. 2014). Or it is a failure of the currently used methods of clinical impact estimating in variants in situations without clear clinical correlation.

The presented clinical data implies that the detected variants induce an aberrant phenotype via increased activation of FGFR3 even with its low expression. In such a case, low molecular weight drugs (e.g. tubacin) could be used to suppress clinical symptoms (Ota 2017).

Growth hormone receptor (*GHR*)

GHR-receptor-mediated cell signalling in response to growth hormones in hepatocytes is important for many physiological processes including growth regulation. The activation of several intracellular pathways via a ligand binding to the receptor is very well described (e.g. JAK-STAT signalling). Other factors are not understood well (e.g. activation of SRC family kinases) (Chhabra Y et al. 2021). Clinically, pathogenic variants in the *GHR* gene are most commonly associated with Laron syndrome (LS, OMIM: 262500). Endocrinologically, LS is characterised by very short stature (mean -6.8 SDS), normal or high GH levels, and very low serum IGF-1 and IGFBP-3 levels. Other important clinical signs of LS include a small face, high-pitched voice, normal body proportions in childhood, but growth restriction worsening in later stages. Delayed bone age and occasionally blue sclera and hip degeneration are reported in

adults. The cause of such a severe phenotype is homozygous function loss of the *GHR* gene (Laron Z et al. 1974, Laron Z et al. 2004, Al-Ashwal AA et al. 2017). Nonetheless, heterozygous variants in the *GHR* gene have a milder clinical effect – reduced or, conversely, increased sensitivity to growth hormone is usually reported (OMIM: 604271). While reduced GH sensitivity is very rarely reported and usually in dated literature, increased GH sensitivity occurs as European polymorphism consisting of a genomic deletion of exon 3 (d3GHR) (Goddard AD et al. 1995, Walker JL et al. 1998, Pantel J et al. 2000). This variant was also observed in our laboratory. However, it was excluded from candidate variants due to the high frequency of occurrence. Moreover, the available literature points to its role in specific diseases, where it may or may not modulate the effect of the treatment, rather than leading to an aberrant phenotype by itself (Szmit-Domagalska J et al. 2016, Chiloiro S et al. 2018, Bianchi A et al. 2019). This study identified two suspected heterozygous variants in *GHR*.

No relevant phenotype has been described for the heterozygous c.440-1G>A variant as of now. Our study describes a specific phenotype of a patient with this variant. The boy's height is reduced by 1.46 SDS when compared with the average and is accompanied by delayed bone age. The patient also has facial dysmorphic features and short toes. He also underwent physiotherapy due to delayed psychomotor development until 13 months of age. c.440-1G>A is expected to disrupt RNA splicing, which leads to the loss of protein function (Baralle D et al. 2005). This variant was also observed in homozygous condition, leading to the development of LS. Therefore, the ClinVar database classifies it as pathogenic (Amselem S et al. 1993, Al-Ashwal AA et al. 2017). This classification is confirmed in this study based on phenotypic manifestations similar to those of patients with reduced sensitivity to growth hormone and also based on the co-segregation of the variant with the phenotype. The variant was inherited from the mother of the patient with similar clinical features and a height of 160 cm. The inheritance is consistent with to-date knowledge in this case. Her height falls within the estimated values calculated from the height of her grandparents (149-166 cm). Unfortunately, it was not possible to perform genetic analysis of the proband's maternal grandmother, who measures 158 cm. Therefore, the calculation may be misleading.

Our study also detected a heterozygous variant c.432T>G, which leads to amino acid substitution (p.Asp144Glu). The prediction tool considered this substitution as probably damaging to the resulting protein. At of now, no reports of this variant were be found, so it is considered a novel finding. Some similarities may be observed between the carrier of c.432T>G and the patient with the pathogenic c.440-1G>A variant. Another c.432T>G carrier was a boy with a shorter stature by 1.93 SDS when compared with the average and delayed bone age. Nonetheless, the remaining phenotypic aberrations did not match the previous patient. We consider this variant to be of unclear clinical significance due to the lack of further evidence.

The confirmation of pathogenic variants in *GHR* is especially important to meet the indication criteria for the treatment, e.g., with rhIGF-1 (mecasermin), where 80% of patients with LS achieve the correct growth rate. It may therefore be assumed that it could help patients with reduced growth hormone sensitivity caused by heterozygous changes in the *GHR* gene (Kamil G et al. 2023).

Collagen type II alpha 1 chain (*COL2A1*)

Type I collagen is the most abundant and ubiquitously distributed of the collagen protein family (Dalgleish R et al. 1997). The three chains of its protein structure are folded together to form a triple-helical configuration and procollagen homotrimer (Rani 1999). The triple helix structure was confirmed via X-ray crystallographic studies (Bella J et al. 1994). The described structure contains glycine molecules as every third residue along the entire length of the triple helix. Its substitution or loss is quite common in the protein structure, but each alteration exerts a different effect on the resulting protein. Glycine substitutions were confirmed the least disruptive, whereas deletions were determined to lead to the most severe damage (Long CG et al. 1993).

In our study, the heterozygous variant c.1636G>A, leading to glycine substitution (p.Gly546Ser) was detected. It was previously described in two Chinese families with congenital spondyloepiphyseal dysplasia (SEDC, OMIM: 183900) (Xu 2014, Chen J et al. 2017). SEDC symptoms usually include dwarfism, short trunk, flat face, platyspondylosis with irregular endplates, short neck, delayed epiphyseal ossification and barrel chest (Xu 2014, Terhal 2015). The height of the members of the reported family with the c.1636G>A variant did not exceed 143.2 cm (Xu 2014, Chen J et al. 2017).

A girl from our group inherited the pathogenic variant c.1636G>A from her father, who is of below average height (160 cm, https://www.worlddata.info/average-bodyheight.php 12/2/2022). However, the phenotype of neither him nor the girl matched SEDC. Previously reported cases originated from Asia, but our family is European. The clinical picture of this family rather corresponds to osteoarthritis with mild chondrodysplasia (OMIM: 604864). This disease leads to reduced height in the carriers of a pathogenic variant in *COL2A1*, when compared to siblings without the variant. The father of the subject is 160 cm, but his brother is 174 cm. The height predicted based on the height of the father's parents was 165-182 cm. Furthermore, the father was treated for Scheuermann's disease (OMIM: 181440) characterised by lumbar or thoracic kyphosis or both, back pain and a variety of vertebral changes including wedging, endplate irregularity and narrowing of the spaces between the discs (Axenovich TI et al. 2001). This fits the clinical description of osteoarthritis with mild chondrodysplasia (OMIM: irregular endplates, mild platyspondylia, Schmorl's nodes, anterior wedging). The exact pathophysiology of Scheuermann's disease is still unknown. However, it is very likely that it is also of genetic aetiology (Mansfield JT et al. 2023). In conclusion, we confirmed the variant c.1636G>A as pathogenic with autosomal dominant inheritance which is concordant with all diseases caused by the pathogenic variant in *COL2A1*. However, SEDC did not develop in this case.

Suspect variants of clinically unclear significance in the *COL2A1* gene were also detected in this study Two of them are located in the 5'UTR region (c.*111C>T, c.*231C>A) and one in the intron near the splicing site (c.1680+8_1680+9delGCinsTA). Both 5'UTR variants were predicted to damage the resulting protein with high probability. One class of neglected non-coding variations are the 5'UTR variants leading to a change in the regions for reading frame opening (the so-called upstream open reading frames, upORF). This may result in a decrease in the expression of the RNA gene and thus the protein (Soukarieh O et al. 2022). Both carriers of these variants were boys with reduced height by 1.79-1.98 SDS, but their other clinical features differed substantially. The c.1680+8_1680+9delGCinsTA variant was also of interest. It is currently described separately in the databases (c.1680+8G>T and c.1680+9C>A). However, it was also a finding stemming from one sample, not two findings in two different patients (ClinVar, Variant ID 308923, 308924). None of the records contained clinical data for this variant, but it is considered of unclear clinical significance or benign. According to the prediction the c.1680+8G>T substitution should be pathogenic and the c.1680+9C>A benign. Our subject showed only mild reduction in height (-0.93 SDS) with no other substantial clinical features. She underwent genetic testing, mainly due to the positive family history. We considered all three variants as clinically unclear due to the lack of information.

Short stature homeobox (*SHOX*)

Our study also detected variants in the *SHOX* gene. The pathogenic role of its gene aberrations is well known. Disorders associated with these alterations are labelled Langer mesomelic dysplasia (homozygous loss of SHOX, OMIM: 249700) and Leri-Weill dyschondrosteosis (heterozygous loss of SHOX, OMIM: 127300) (Langer LO 1967, Leri, A and Weill, J. 1929). This gene was also reported in association with short stature (OMIM: 300582). The clinical features of pathogenic variant carriers of the gene often include Madelung deformity of the forearm. Furthermore, earlier studies reported a similar clinical phenotype in patients with *SHOX* regulator deletions. Regulators are highly conserved sequences around a gene and their loss may cause a similar or identical phenotype as the loss of the

gene itself (Benito-Sanz S et al. 2012, Rappold GA et al. 2002, Chen et al. 2009, Bunyan et al. 2014, Seki et al. 2014, Hands JM et al. 2022).

A girl from our group was a carrier of a heterozygous loss of g.(?_780700)_(835572_?)del overlapping the CNE7, CNE8 and CNE9 regulators. Her growth restriction was not significant (-0.9 SDS) and her figure was proportional without substantial dysmorphic features. However, she underwent a surgical intervention due to bilateral Madelung deformity at a younger age. The origin of this variant has not been determined, but the phenotype of her parents does not point to the presence of the variant. We consider the variant to be pathogenic due to the presence of Madelung deformity, which is typical in the aberrations of *SHOX* or its regulatory regions. Nonetheless, it is listed as clinically unclear in the ClinVar database.

Furthermore, we detected c.-9delG in the *SHOX* gene in a patient with -1.66 SD. Although the prediction tool considered this variant to be benign and some authors report it in the same way, a relatively recent functional study demonstrated a decrease in *SHOX* expression in cells with a homozygous form of c.-9delG (Babu et al. 020, Hirschfeldova et al. 2017). The variant was present in the heterozygous state in our study, with a frequency significantly higher than in the general population. The laboratory's internal documentation showed the capture in 1:500, which corresponds to the capture rate in a group of Brazilian children with short stature (Babu et al. 2020). The pathogenicity of this variant cannot be reliably confirmed. Hence, this question requires further investigation and clinical evidence.

Aggrecan (*ACAN*), Insulin like growth factor binding protein acid labile subunit (*IGFALS*), Insulin like growth factor 1 receptor (*IGF1R*)

Suspicious variants were identified in the *ACAN, IGFALS, IGF1R, NPR2* genes possibly associated with the development of congenital skeletal aberrations. Although some of them occurred repeatedly in unrelated patients (*IGF1R* c.1247+3A>G three times, *IGFALS* c.179C>T twice), the phenotypes often differed. The demonstrated small stature (< -2 SDS), which should be one of the most important clues for the diagnosis of congenital bone aberrations, was only identified in the c.206T>A variants in the *ACAN* gene, c.179C>T in the *IGFALS* gene and in one of the c.1247+3A>G carriers in the *IGF1R* gene (Geister KA et al. 2015, Chiarelli F et al. 2020). Pathogenic variants of *ACAN* are associated with the development of short stature with accelerated bone age with/without early osteoarthritis (OMIM 165800) and with aggrecan-type spondyloepimetaphyseal dysplasia (OMIM: 612813). Although the patient has a significantly shorter stature (-3.13 SDS), other clinical features do not correspond to any of the described syndromes. A repeat variant in the *IGFALS* gene also cannot be considered the cause of the lack of the acid-labile subunit (OMIM: 615961) because it is an autosomal recessive disease, but our patients were heterozygous c.179C>T carriers.

The c.1247+3A>G variant in the *IGF1R* gene was very interesting for the clinical-genetic correlation. Although we only had clinical data from two of the three carriers of this variant, both carriers had low height (-1.89, -2.11 SDS) and delayed bone maturation. Resistance to IGF1 has been clinically reported (OMIM: 270450) with a similar phenotype. Less conspicuous clinical specifics of this disease were only present in one of our patients (clinodactyly, short fingers, smooth philtrum, low-set ears, wide nasal bridge). Insulin-like growth factors (IGFs) and their receptors play a central role in the chondrogenesis and deviations in them have been associated with the development of skeletal aberrations (Spagnoli A et al. 2005, De Luca F 2018).

The last gene with identified suspect variants is *NPR2*. Unfortunately, clinical data was only available for one of the patients. This receptor for natriuretic peptides plays an important role in complex paracrine regulation of the growth plate. The signalling cascade of the receptor promotes bone matrix synthesis and stimulates cartilage proliferation and differentiation. Heterozygous variants in the *NPR2*

gene have been reported in 2-6% of cases with idiopathic short stature (Stavber L et al. 2022, Yuan K et al. 2021, Ke X et al. 2021). This study reports on a patient with c.1711-8delT, leading to thymine deletion in a region close to the post-transcriptional splice site. The Predict SNP2 prediction tool is not able to calculate the pathogenicity probability of deletions. Therefore, no damaging:benign ratio is provided. However, this variant has not been previously described. The ClinVar database only lists the c.1711-9T>G substitution with unclear clinical significance associated with Acromesomelic dysplasia 1, Maroteaux type (OMIM: 602875). This disease is caused by homozygous loss of receptor function and the relevant clinical picture does not fit our patient. On the other hand, small stature with non-specific skeletal abnormalities has also been described (OMIM: 616255), with autosomal dominant inheritance and proportionally small stature (-1.0 to -3.0 SDS). Patients are phenotypically unremarkable apart from reduced height (Olney RC et al. 2005, Vasques GA et al. 2013, Amano N et al. 2014). Our patient fits this phenotype better. Unfortunately, we were not able to determine the origin of the variant. Therefore, it is considered of unclear clinical significance.

Conclusion and our opinion on the context of the results

As the majority of patients with congenital bone aberrations are of borderline or small stature associated with other clinical problems, therapeutic management is addressed (Geister KA et al. 2015, Chiarelli F et al. 2020). Knowing the genetic make-up of the individual is important for deciding the most appropriate therapy. Treatment with a recombinant growth hormone (rhGH) is likely to be less effective than the administration of recombinant insulin-like growth factor 1 (rhIGF1) in patients with pathogenic variants in the GHR, IGFALS, IGF1 genes (Bright et al. 2016). The information about the genetic cause of bone aberrations is also important for pregnancy planning in families with pathogenic variants. Some of the more severe phenotypes associated with these variants only lead to relevant phenotypes in homozygous form. Genetic counselling and a possible carrier test are recommended in heterozygous carriers with mild phenotype, which may not be detected in all the cases (Guo L et al. 2023, Murrin EM et al. 2022, Savarirayan R et al. 2018). We are aware that the selection of the genes and design of the NGS panel used in the study is not exhaustive which is a limitation of the study. The following functional studies are necessary to confirm our findings and to resolve whether these variants actually cause the disease or are involved in the disease process. However, we hope that our results may extend present knowledge and initiate further research.

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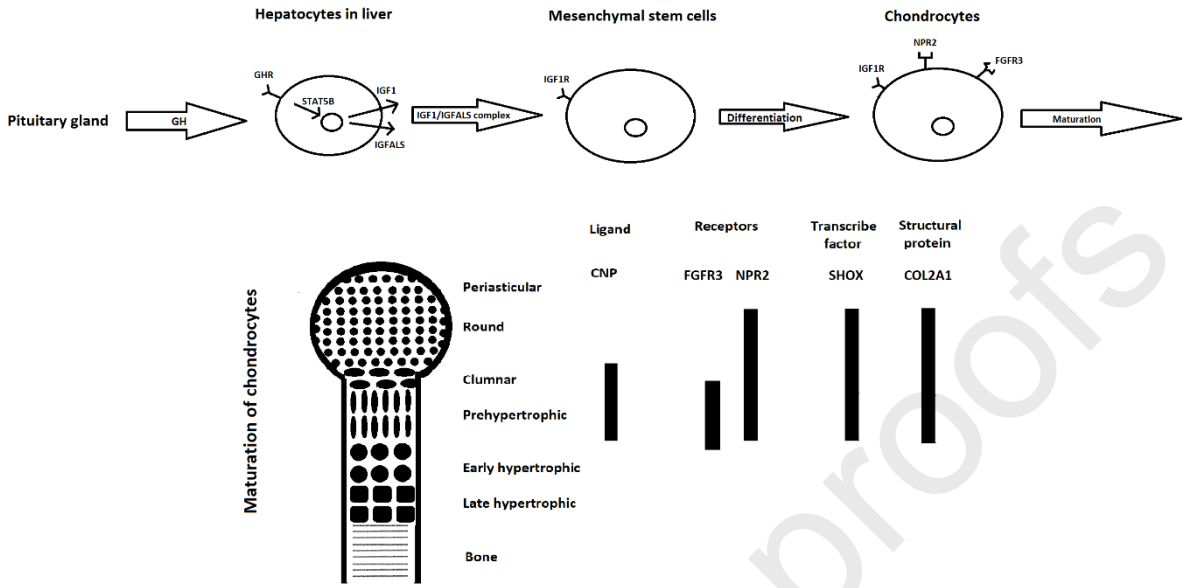
Figures

Figure 1: Roles of selected genes in bone growth



Schematic representation of growth hormone (GH)–GH receptor (GHR)–insulin-like growth factor 1 (IGF1) axis. GH is produced by the pituitary gland. GH combines with GHR to regulate the production of IGF1 (Hu et al 2021). GH binds its receptor and then activates Jak2, which in turn activates STAT5 by phosphorylation. Phosphorylated STAT5 goes to the nucleus, where it activates transcription of the *IGF1* and *IGFALS (*Baik M et al 2011). IGF-1 contributes to the chondrogenic differentiation of mesenchymal stem cells (MSCs). Also, IGF-1 promotes proteoglycan synthesis in chondrocytes by activating the PI3K pathway and stimulates chondrocyte proliferation via the PI3K and MEK/ERK pathways (Wen C et al 2021). CNP and its receptor, the guanylyl cyclase natriuretic peptide receptor 2 (NPR2), are expressed in chondrocytes as well as in osteoblasts and are recognized as important regulators of longitudinal bone growth and bone homeostasis (Shuhaibar LC et al 2021).

Figure 2: Family tree of the carrier of c.1657G>A in *FGFR3*

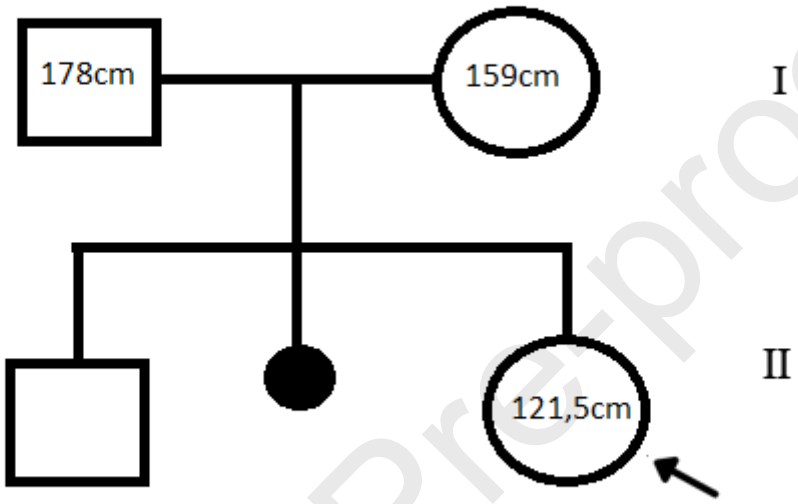


Figure 3: Family tree of the carrier of c.2005C>G in *FGFR3*

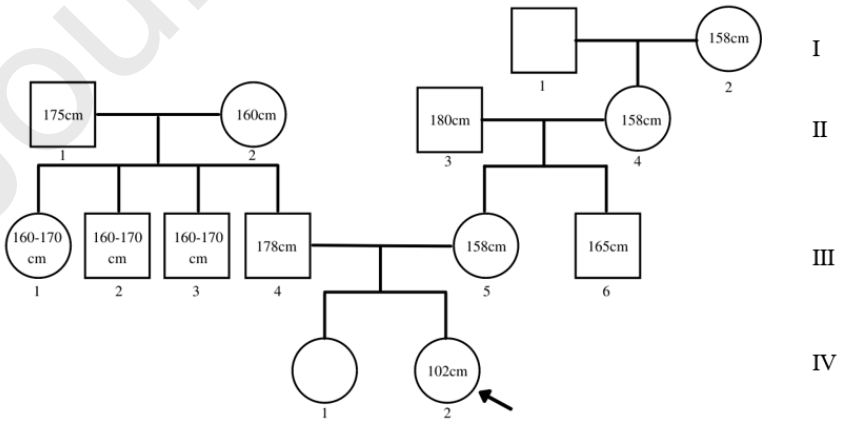


Figure 4: Family tree of the carrier of c.440-1G>A in *GHR*

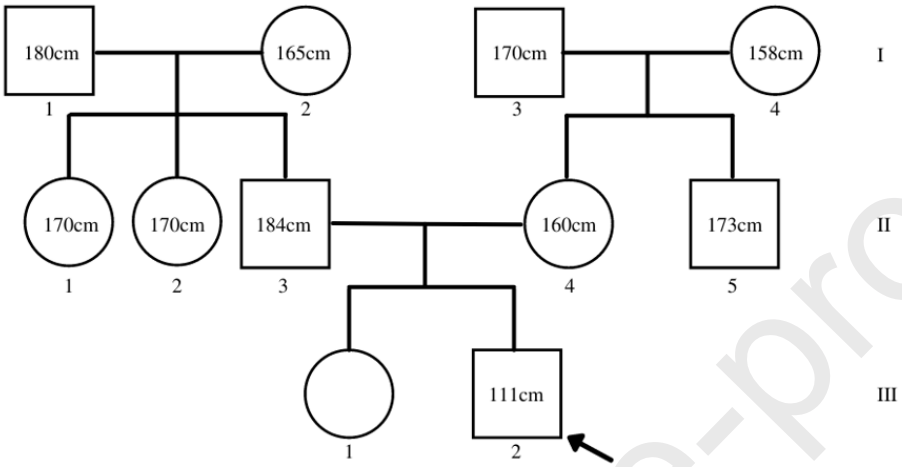


Figure 5: Family tree of c.1636G>A in *COL2A1*

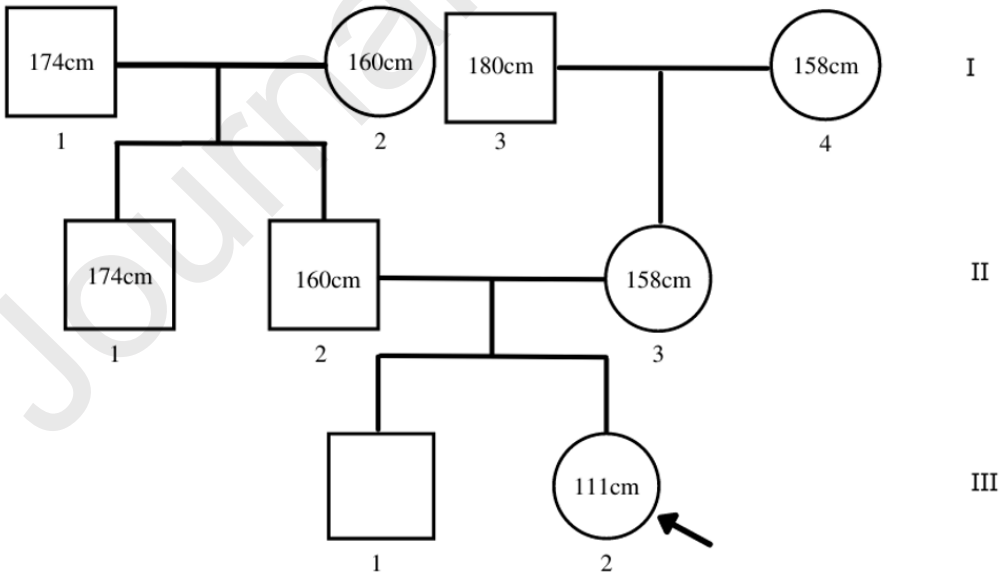
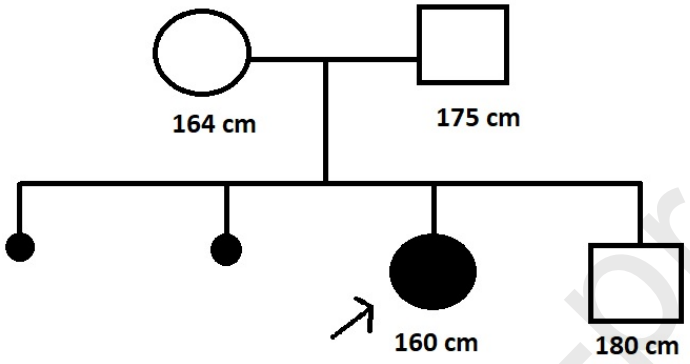


Figure 6: Family tree of the carrier of NC_000023.11:g.(?_780700)_(835572_?)del in *SHOX*



Tables

Table 1: Areas not covered

Gene	Reference transcription	Exon
ACAN	NM_001369268.1	13
		14
		18
FGFR3	NM_001163213.1	8
GHR	NM_1242399.2	1
IGF1	NM_000618.5	4

	NM_001111284.2	1
SHOX	NM_000451.3	6
	NM_006883.2	6

Table 2: Nucleotide primer sequences for Sanger sequencing

Reference transcription	Sequence
NM_000451.3	forward: TAGGGGAGAAGAGGCACGTTG
	reverse: GAAGGAGCTCCAGGCGGGGTTG
NM_006883.2	forward: ATTGATGGTTAGTATTTTTTGTAGCAGTTG
	reverse: TTAAAAATAAAGTTACAAAGGCCGGG
NM_000142.4:c.2005C>G	forward: GAAGATCGCAGACTTCGGGC
	reverse: CCCAGGGAGGGGTAGAAACC
NM_000163.5:c.440-1G>A	forward: TCCAAAAGGAATTTTTGTAAGGGC
	reverse: ATGGAGCATACAGCATGAACA
NM_001844.5:c.1636G>A	forward: CATCGCTGAGGTCACATGGT
	reverse: CCTCCCAATCCTGGCAGTG
NM_000142.5:c.943A>C	forward: CGGGAAACACAAAAACATCATCAAC
	reverse: GCTGAAGCCTCTCCACCTCTC

NM_000142.5:c.1657G>A	forward: CACGACCTGTGAGTGGCAT
	reverse: GGGGAGTACTGCTCGAAAGG

Table 3: Pathogenic variants and overview of their carriers' clinical data

Gene	*FGFR3*	*FGFR3*	*FGFR3*	*GHR*	*COL2A1*	*SHOX*
Reference transcription	NM_000142.5	NM_000142.5	NM_000142.4	NM_000163.5	NM_001844.5	NC_000023.11
HGVS	c.943A>C	c.1657G>A	c.2005C>G	c.440-1G>A	c.1636G>A	g.(?_780700)_(835572_?)del
Variant frequency in non-Finnish European population	0,000009	0,000063	0,000004	0,000018	N/A	N/A
Amino acid substitution	p.Asn315Asp	p.Val553Met	p.Arg669Gly	splice site	p.Gly546Ser	
Protein domain	Ig-like C2-type 3	Protein kinase	Protein kinase	Fibronectin type-III	Triple-helical region	Regulator of SHOX
ClinVar classification	unclassified	of unclear clinical significance	of unclear clinical significance	pathogenic	pathogenic	of unclear clinical significance
Origin	*de novo*	pat.	mat.	mat.	pat.	N/A
Sex	M	F	F	M	F	F
Age (months)	159	118	63	77	88	167
Height (cm)	156,8	121,5	102,0	111,0	111,0	160,0
Z-score	-0,04	-2,53	-1,79	-1,46	-2,10	-0,96
Weight (kg)	56,4	24,4	16,7	16,7	26,3	56
Head circumference (cm)	N/A	51,5	51	52	51,5	53,5
Facial dysmorphism	yes	no	yes 2)	yes 3)	no 4)	no 5)
Disproportionate stature	yes	no	yes	no	no 4)	no
PMR	yes	no	no	yes 3)	no	no
IUGR	no	no	no	no	no	no
Delivery term	40+0	in term	in term	in term 3)	in term	40+0
Delivery metrics	4200 g/51 cm	2700 g/48 cm	3265 g/46 cm	3200 g/49 cm	3830 g/ 48 cm	3330 g/50 cm
Skin abnormalities	no	no	no	yes 3)	no	no
Bone age (months)	delayed	delayed	corresponding to the age	delayed	accelerated	corresponding to the age
Extremities	yes 1)	normal	yes 2)	yes 3)	no	yes 5)
Family history	yes	normal	yes	Yes	Yes	yes 5)
Height of mother (cm):	172	159	158	160	158	164
Height of father (cm):	180	178	178	184	160	175
Endocrinology	no finding	no finding	no finding	no finding	Grade 1 obesity	no finding

1) paternal grandmother of shorter stature; pedes plani
2) Triangular face shape, high wide forehead, hypertelorism, wide nasal root, short philtrum, narrow lips, bluish sclerae, long upper body segment, wide chest, short fingers, short 3rd toe in lower limbs, flat foot bilaterally
3) physiotherapy concluded at the age of 13th months; oval face shape, broad nasal bridge, anti-Mongoloid slant of eye slits, low-set ears, atypical palmar grooves, flat foot bilaterally, short toes, prolonged delivery
4) high forehead, short neck
5) Madelung deformity bilaterally, oval face, higher forehead. Short philtrum, full lips
N/A = unavailable data, pat.

Table 4: Genetic data on unclear variants occurring significantly more often in the studied cohort

Gene	HGVS	Position	Amino acid substitution	Sex	Z-score	Predict SNP2 (damaging:benign)	Number of detections in the population	Number of tested subjects in the population	PhyloP score	ACMG classification	Allele frequency in the European non-Finnish populatio	Allele frequency in the African/American populatio	Allele frequency in the Asian population	Frequency in the studied cohort	p value	Notes
ACAN	NM_013227.3:c.1527G>A	exon	p.Ser509=	M	-3.94	0:5	6	88704	-4,89	probably benign	0,000068	N/A	N/A	0,00775	0,0101	
ACAN	NM_013227.3:c.820C>T	exon	p.Arg274Trp	F	N/A	**3:2**	13	125007	2,24	benign	0,000109	0,000066	0,000996	0,00775	0,0143	
ACAN	NM_013227.3:c.1417C>T	exon	p.His473Tyr	M	-1.93	0:5	1	15429	0,54	probably benign	N/A	N/A	N/A	0,00775	0,0165	
ACAN	NM_013227.3:c.6486C>T	exon	p.Ser2162=	F	-2.11	0:5	17	128291	-6,46	probably benign	0,000142	N/A	0,000033	0,00775	0,0179	
ACAN	NM_013227.3:c.6142C>G	exon	p.Pro2048Ala	M	-1.79	2:3	23	112933	2,23	probably benign	0,000204	N/A	0,000229	0,00775	0,0270	
ACAN	NM_013227.3:c.206T>A	exon	p.Leu69Gln	M	-3.13	2:3	**N/A**	**N/A**	**5,93**	unclear clinical significance	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	
COL2A1	NM_001844.4:c.3642T>C	exon	p.Pro1214=	M	-2.37	0:5	3	127383	-4,24	probably benign	0,000009	N/A	N/A	0,00775	0,0087	
COL2A1	NM_001844.4:c.*231C>A	3'UTR		M	-1.79	**5:0**	1	29117	4,02	probably benign	0,000034	N/A	N/A	0,00775	0,0088	#
COL2A1	NM_001844.4:c.*111C>T	3'UTR		M	-1,98	**5:0**	**N/A**	**N/A**	**3,22**	probably benign	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	
COL2A1	NM_001844.5:c.1680+8_1680+9 delGCinsTA	intron		F	-0,91	N/A	**N/A**	**N/A**	**'-0.30; 0.32**	probably benign	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	*
FGFR3	NM_001163213.1:c.1966-20G>A	intron		M	-2,23	0:5	1	112275	0,03	probably benign	0,000009	0,000124	N/A	0,00775	0,0023	
GHR	NM_001242399.2:c.432T>G	exon	p.Asp144Glu	M	-1,93	**3:2**	**N/A**	**N/A**	**-0,72**	unclear clinical significance	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	
IGF1R	NM_000875.3:c.1194C>T	exon	p.Ser398=	F	-2,42	1:4	125	129071	0,06	probably benign	0,001040	0,000185	0,000122	0,01550	0,0073	
IGF1R	NM_000875.3:c.1194C>T	exon	p.Ser398=	M	-2,08	1:4	125	129071	0,06	probably benign	0,001040	0,000185	0,000122	0,01550	0,0073	
IGF1R	NM_000875.3:c.1247+3A>G	intron		F	-1,89	**4:1**	130	129052	0,86	probably benign	0,000870	0,000062	0,001500	0,01550	0,0078	
IGF1R	NM_000875.3:c.1247+3A>G	intron		F	N/A	**4:1**	130	129052	0,86	probably benign	0,000870	0,000062	0,001500	0,01550	0,0078	
IGF1R	NM_000875.3:c.1247+3A>G	intron		F	-2,11	**4:1**	130	129052	0,86	probably benign	0,000870	0,000062	0,001500	0,01550	0,0078	
IGFALS	NM_001146006.2:c.1319G>A	exon	p.Arg440His	M	-2,08	1:4	6	111166	0,38	probably benign	0,000054	N/A	N/A	0,00775	0,0081	
IGFALS	NM_001146006.1:c.768C>G	exon	p.Ala256=	M	-1,31	0:5	22	74614	-1,33	probably benign	0,000355	N/A	0,000857	0,00775	0,0389	
IGFALS	NM_001146006.1:c.179C>T	exon	p.Pro60Leu	F	-2,14	1:4	3	104286	0,072	probably benign	0,000949	0,000078	0,000110	0,01550	**<<0.05**	
IGFALS	NM_001146006.2:c.179C>T	exon	p.Pro60Leu	M	-2,40	1:4	3	104286	0,072	probably benign	0,000949	0,000078	0,000110	0,01550	**<<0.05**	
IGFALS	NM_001146006.2:c.1817G>T	exon	p.Gly606Val	F	N/A	1:4	**N/A**	**N/A**	**0,99**	unclear clinical significance	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	
NPR2	NM_003995.3:c.1558-18T>C	intron		F	N/A	**5:0**	2	113762	4,32	probably benign	0,000018	N/A	0,000196	0,00775	0,0034	
NPR2	NM_003995.3:c.*209A>G	3'UTR		F	N/A	**3:2**	2	107960	-0,4	unclear clinical significance	0,000019	N/A	N/A	0,00775	0,0036	
NPR2	NM_003995.3:c.1572C>T	exon	p.Tyr524=	M	-2,57	2:3	22	129136	0,87	probably benign	0,000141	0,000062	N/A	0,00775	0,0227	
NPR2	NM_003995.3:c.1711-8delT	intron		F	-1,8	-	**N/A**	**N/A**	**-1,5**	probably benign	**N/A**	**N/A**	**N/A**	0,00775	**N/A**	
SHOX	NM_000451.3:c.544+9C>T	intron		M	-1,76	0:5	2	113462	-0,23	probably benign	0,000018	N/A	N/A	0,00775	0,0034	

*# Frequency of the variant from the Bravo database, * The frequency was listed separately in the gnomAD database = NM_001844.5:c.1680+8G>T and NM_001844.5:c.1680+9C>A, N/A – non-available data*

Table 5: Clinical data of candidate variants in clinically unclear variants

Gene	*ACAN*		*COL2A1*			*GHR*	*IGFALS*			*IGF1R*			*NPR2*		
Transcript	NM_013227.3		NM_001844.4			NM_001242399.2	NM_001146006.1			NM_000875.3			NM_003995.3		
cDNA	c.206T>A	c.820C>T	c.*111C>T	c.*231C>A	c.1680+8_1680+9 delGCinsTA	c.432T>G	c.1817G>T	c.179C>T		c.1247+3A>G			c.1711-8delT	c.*209A>G	c.1558-18T>C
Position	exon	exon	3'UTR	3'UTR	intron	exon	exon	exon	exon	intron	intron	intron	intron	3'UTR	intron
Amino acid substitution	p.Leu69Gln	p.Arg274Trp				p.Asp144Glu	p.Gly606Val	p.Pro60Leu	p.Pro60Leu						
Sex	M	F	M	M	F	M	F	F	M	F	F	F	F	F	F
Age (years)	3,25	N/A	4	13,33	5,75	5	N/A	8,17	4,67	2,74	N/A	6,33	4,33	N/A	N/A
Height of patient (cm)	86	N/A	94,5	145	109	101	N/A	115	97	86	N/A	106	96	N/A	N/A
Z-score	-3.13	N/A	-1,98	109	-0,93	-1,93	N/A	-2,14	-2,40	-1,89	N/A	-2,11	-1,8	N/A	N/A
Weight (kg)	10,9	N/A	13,7	31,8	18,7	14,3	N/A	17,9	12	10	N/A	15,5	13,2	N/A	N/A
Head circumference (cm)	48	N/A	49	55	49,5	50	N/A	51	48	48	N/A	50	48	N/A	N/A
Facial dysmorphism	no	N/A	yes 1)	no	no	no	N/A	no	yes 5)	yes 3)	N/A	no	no	N/A	N/A
Disproportionate stature	no	N/A	no	no	no	no	N/A	yes 4)	no	yes 3)	N/A	no	no	N/A	N/A
PMR	no	N/A	no	yes 6)	no	no	N/A	yes 4)	no	no	N/A	no	no	N/A	N/A
IUGR	no	N/A	no	no	no	no	N/A	no	YES	no	N/A	no	YES	N/A	N/A
Delivery term	40+2	N/A	39+0	41+0	41+0	38+6	N/A	38+0	40+4	N/A *	N/A	N/A *	N/A *	N/A	N/A
Delivery metrics	2670 g/49 cm	N/A	3333 g/51 cm	3150 g/47 cm	3885 g/48 cm	NA	N/A	2830 g/49 cm	2660 g/49 cm	2600 g/48 cm	N/A	2800 g/48 cm	2300 g/43 cm	N/A	N/A
Skin abnormalities	no	N/A	no	no	no	no	N/A	no	no	no	N/A	no	no	N/A	N/A
Bone age	delayed	N/A	NA	delayed	N/A	delayed	N/A	N/A	delayed	delayed	N/A	delayed	normal	N/A	N/A
Extremities	no	N/A	yes 1)	yes 6)	no	no	N/A	yes 4)	yes 5)	yes 3)	N/A	no	yes 2)	N/A	N/A
Family history	no	N/A	yes 1)	no	yes 7)	no	N/A	yes 4)	yes 5)	no	N/A	N/A	no	N/A	N/A
Height of mother (cm):	158	N/A	156	164	168	168	N/A	165	144	160	N/A	155	165	N/A	N/A
Height of father (cm):	177	N/A	175	167	170	175	N/A	180	170	178	N/A	165	168	N/A	N/A
Endocrinology				yes 6)				yes 4)	yes 5)						

1) triangular face, hypertelorism, marked epicanthic folds bilaterally, wide palpebral fissures, *irides stellatae* , wide root of nose, raised tip of nose with bulbous appearance, marginal philtrum, full lips; short toes, nails without prominence, flat foot bilaterally, short toes, mild sandal groove bilaterally; mother's father was treated with growth hormone in childhood due to short stature

2) flat foot bilaterally, sandal groove bilaterally

3) hypertelorism, broad nasal bridge, smoothed philtrum, small mouth, narrow lips, receding chin, high-arched palate, low-set ears, larger auricles, long upper body, short fingers, mild clinodactyly of the little finger, bending foot area, big toes in lower limbs bent; no ossifying nucleus of the little finger

4) The stature is mildly disproportionate; *pedes plani* ; developmental dysphasia, hypermobility syndrome; father was short until the age of about 10 years; Isolated growth hormone deficiency, other components of hypopituitarism unproven, MRI – hypothalamo-pituitary region normal, GH treatment, growth rate significantly improved by treatment.

5) epicanthic folds, long philtrum, large auricles; mild clinodactyly of little fingers bilaterally; mother is 144 cm tall. Madelung deformity, mesomelia indicated. Also a shortened forearm; borderline IGF1

6) congenital hypotonia – physiotherapy within 1 year; *pedes plani* ; borderline IGF1, normal when corrected for the delayed biological level of maturation

7) sister, father's brother, father's parents, father's grandmother from the mother's side – 3rd percentile for growth

* full term delivery

N/A – unavailable data; PMR – psychomotor restriction; IUGR – intrauterine growth restriction; M – boy; F – girl

Clinical-genetic analysis of selected genes involved in the development of the human skeleton in 128 Czech patients with suspected congenital skeletal abnormalities

- Detection of pathogenic variants in genes associated with congenital skeletal abnormalities.
- Detailed clinical description of detected pathogenic variants.
- Genotype-phenotype correlation and segregation of pathogenic variants.
- Better understanding of the skeletal pathophysiology.
- Spectrum of pathogenic variants associated with the development of the human skeleton in Czechia.

Clinical-genetic analysis of selected genes involved in the development of the human skeleton in 128 Czech patients with suspected congenital skeletal abnormalities

Abbreviations list

ACAN - Aggrecan

CNVs - copy number variants

COL2A1 - Collagen type II alpha 1 chain

FGFR3 - Fibroblast growth factor receptor 3

GHR - Growth hormone receptor

IGFALS - Insulin like growth factor binding protein acid labile subunit

IGF1R - Insulin like growth factor 1 receptor

MLPA - Multiplex Ligation-dependent Probe Amplification

NGS - Next Generation Sequence

SDS - standard deviation score

SNVs - single nucleotide variants

SHOX - Short stature homeobox

WHO - World Health Organization

Clinical-genetic analysis of selected genes involved in the development of the human skeleton in 128 Czech patients with suspected congenital skeletal abnormalities

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: