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Retrospective Analysis of Multi-Method Sequencing Results in Patients with Skeletal Dysplasia

İskelet Displazisi Olan Hastalarda Çok Metotlu Dizileme Sonuçlarının Retrospektif Analizi

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ABSTRACT

Objective: Skeletal dysplasia is a heterogeneous disease affecting the development of the skeletal system. Due to its complexity, clinical or radiological examinations can be informative; therefore, molecular approaches may be more effective for differential diagnosis and may assist in clarifying subtypes. Although studies in cohorts have identified associated genes and variants, further research is needed. This study aims to investigate the genotype-phenotype relationship in a Turkish cohort.

Methods: Thirty-six patients were involved in the study. Patients were classified according to the 2023 revision of the Nosology of Genetic Diseases of the Skeletal System. Following the genetic diagnostic algorithm, Sanger sequencing, targeted gene panels, clinical exome sequencing, or whole exome sequencing were performed. The existing literature was reviewed for some of the identified variants.

Results: The proportion of females (63.9%) was higher than that of males (36.1%). Notably, the parents of 16 patients (45.7%) were consanguineous. Pathogenic or likely pathogenic variants were identified in 29 of 36 patients, while variants of uncertain significance were identified in 6 patients. The diagnostic yield was 80% based on sequencing methods. The groups with the highest number of molecular diagnoses were group 2, type II collagen disorders (5 patients with COL2A1 variants), and group 34, syndromes with craniosynostosis (5 patients with FGFR2, FGFR3, TWIST1, or SMO variants), according to nosology.

ÖZ

Amaç: İskelet displazisi, iskelet sisteminin gelişimini etkileyen heterojen hastalık gurubudur. Kompleks doğası nedeniyle, klinik ya da radyolojik değerlendirmeler bilgi verici olabilir; ancak moleküler yaklaşımlar ayırıcı tanı ve alt tiplerin belirlenmesi açısından daha etkilidir. Kohort çalışmaları ilişkili genleri ve varyantları aydınlatmış olsa da bu konuda halen çalışmaya ihtiyaç duyulmaktadır. Sunulan çalışma bir Türk kohortunda genotip-fenotip ilişkisini araştırmayı amaçlamaktadır.

Yöntemler: Sunulan çalışmaya 36 hasta dahil edilmiştir. Hastalar, Genetik İskelet Hastalıklarının Nozolojisi, 2023'e göre gruplandırılmıştır. Genetik tanı algoritmasına uygun olarak hastalara Sanger dizileme, hedefli gen paneli, klinik ekzom dizileme ya da tüm ekzom dizileme çalışılmıştır. Tespit edilen spesifik varyantlar için mevcut literatür derlenmiştir.

Bulgular: Katılımcılar arasında kadın sayısı (%63,9), erken sayısından (%36,1) daha fazladır. Kritik olarak, 16 hastanın (%45,7) ebeveynleri akrabadır. Otuz altı hastanın 29'unda patojenik ya da olası patojenik varyant tespit edilmiştir. Dizileme metoduna bağlı olarak tanı verimi %80'dir. Nozolojiye göre moleküler tanısı en yüksek olan gruplar, grup 2, tip II kolajen hastalıkları (5 hastada COL2A1 varyantları) ve grup 34, kraniosinostozlu sendromlar (5 hastada FGFR2, FGFR3, TWIST1 ya da SMO varyantları) şeklindedir.

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ABSTRACT

Conclusion: This study emphasizes the genetic and phenotypic variation in skeletal dysplasia within a Turkish cohort. We expect these findings to contribute to the current literature and help clinicians in patient follow-up and assessment.

Keywords: Skeletal dysplasia, molecular diagnosis, sequencing

ÖZ

Sonuç: Sunulan çalışma, bir Türk kohortunda, iskelet displazisinde genetik ve fenotipik varyasyonları vurgulamıştır. Elde edilen bulguların mevcut literatüre katkı sunması ve hasta takibi ve değerlendirmesi açısından klinisyenleri yönlendirmesi beklenmektedir.

Anahtar Sözcükler: İskelet displazisi, moleküler tanı, dizileme

INTRODUCTION

Skeletal dysplasia encompasses a wide range of genetic disorders involving abnormalities in bone and cartilage resulting from defects in skeletal development and maintenance (1). It is highly heterogeneous, with more than 450 diseases or syndromes affecting bone growth. In addition to bones and cartilage, skeletal dysplasia can negatively affect muscles, tendons, and ligaments (2,3). Although isolated cases are rare, the overall occurrence of skeletal dysplasia is approximately one in 5,000 people worldwide (2-4).

At the molecular level, various pathways involved in regulating structural proteins, metabolic processes, and the epiphysis play critical roles in the development of skeletal dysplasia (3,5). According to the 2023 revision of the Nosology of Genetic Skeletal Disorders, these conditions are classified into 41 categories, with 552 genes associated with them. Therefore, beyond standard clinical and radiological diagnostic methods, molecular testing can be a valuable tool for differential and definitive diagnosis of skeletal dysplasia. Among these testing methods, gene panels and exome sequencing (ES) are important not only for diagnosing but also for identifying new genes or pathways related to skeletal dysplasia (6,7).

Given the rise of next-generation sequencing (NGS) for disease diagnosis, numerous studies in diverse populations have documented variant frequencies and identified novel or potentially disease-related genes (8-10). However, these efforts still need to confirm variant classifications, especially for those of unknown significance, and to discover new variants in the associated genes by screening diverse cohorts. Therefore, this study analyzed data from Sanger sequencing, gene panels, and ES from 36 patients with skeletal dysplasia. Our results show the usefulness of these methods within a genetic algorithm for the molecular and differential diagnosis of skeletal dysplasia.

MATERIALS AND METHODS

Ethics

The present study was approved by the Ethics Committee of Gazi University (approval number 403; dated 30 May 2022). Both verbal and written consent was obtained from patients or their guardians before the study was initiated.

Participants

The present study involved 36 patients with skeletal dysplasia who were enrolled at the Department of Medical Genetics, Faculty of Medicine, Gazi University in Ankara, between July 2017 and January 2022. Patients were classified according to the 2023 revision of the Nosology of Genetic Skeletal Disorders. Patients with mild phenotypes, including isolated short stature, were excluded from the study.

Genetic Analyses

The disease-related genes were sequenced, and a genetic algorithm-based diagnostic approach was applied. Accordingly, patients with specific skeletal dysplasias who had known genetic mutations in siblings and/or parents were screened using Sanger sequencing. In other cases, NGS using gene panels, clinical ES (CES), or whole-ES (WES) was performed to comprehensively analyze genes associated with skeletal dysplasia phenotypes. All sequencing procedures were carried out after isolating genomic DNA from peripheral venous blood samples using the Qiagen EZ1 Advanced XL automated system (Qiagen Inc., Switzerland), following the manufacturer's standard protocol. The integrity of the isolated DNA was verified by 1% agarose gel electrophoresis, and concentrations were measured using a NanoDrop (NanoDrop 1000; Thermo Scientific, USA) or a Qubit 4.0 (Thermo Scientific, USA).

Sanger sequencing was performed after amplification of specific gene regions containing the relevant variants and further processing using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific, USA). All amplicons were run on the Applied Biosystems 3130 Genetic Analyzer (Thermo Scientific, USA). Finally, chromatogram files were visualized using Chromas 2.6.6 (Technelysium Pty Ltd, Australia), and relevant variants were manually screened.

For the NGS gene-panel application, amplification-based custom panels were used. After single or multiplex amplification of target genes, the amplicons were processed with the Nextera XT DNA Library Preparation Kit and sequenced on the MiSeq System (Illumina Inc., USA). For CES and WES, target regions, including intron–exon boundaries and entire exons, were identified using hybridization-based methods: the SOPHiA Clinical Exome Solution (Sophia Genetics, France) for CES and the Whole Exome Sequencing Panel—Exome 2.0 (Twist Biosciences, USA) for WES. All amplicons were sequenced on either an MGISEQ-2000 (BGI Inc., China) or a NovaSeq 6000 (Illumina Inc., USA) platform.

NGS data were processed using either the Variant Annotation and Filter Tool (VarAft) v2.17.1 (11) or Genomize-SEQ (Genomize, Türkiye). Using these programs, raw FASTQ files were aligned to the human reference genome (hg19) and a binary alignment map file was generated. Next, variant call format files were produced. Using the same tools, variants were filtered based on minor allele frequencies of less than 0.001 in common genome databases and on genes listed in the Human Phenotype Ontology (https://hpo.jax.org/) related to disease. Finally, variants were prioritized and classified using VarSome (https://varsome.com/) or Franklin by Genoox (https://franklin.genoox.com/clinical-db/home), in accordance with the American College of Medical Genetics (ACMG) Guidelines (12).

RESULTS

Clinical Findings

The current study involved 36 patients with skeletal dysplasia. The patients were classified according to the 2023 revision of the Nosology of Genetic Diseases of the Skeletal System and assigned to 16 groups (Table 1). Participants' ages ranged from newborn to 69 years. The proportion of females (23, 63.9%) was higher than that of males (13, 36.1%). The parents of 16 of 36 patients (44.4%) were consanguineous, and 16 of 36 patients (44.4%) had family members with similar clinical features.

Analysis of height measurements in patients within the pathogenic/likely pathogenic (LP) group showed that six individuals had heights above the 3rd percentile at the time of evaluation. These patients were diagnosed with conditions such as Stickler syndrome, Keutel syndrome, osteopetrosis, Shwachman-Diamond syndrome, and craniosynostosis, for which short stature is not typically expected. Although short stature can occur in Shwachman-Diamond syndrome, the height of patient P15 was recorded at the 14th percentile, and data on other indicators, such as growth velocity and bone age, were unavailable. Among five patients with craniosynostosis syndromes, primarily characterized by craniofacial features, two had heights below -3 SD. One of the remaining 25 patients lacked height data; this patient was diagnosed with osteogenesis imperfecta primarily on the basis of recurrent fractures. Among the 18 patients with skeletal dysplasia, in whom short stature was a key feature, 12 had heights below -3 SD and 8 had heights between -3 and -2 SD. A summary of the clinical findings of these patients is shown in Supplementary Table 1.

Molecular Findings

Sequencing results showed that all patients except one (P30, 35/36, 97.2%) had a variant associated with skeletal dysplasia. Of these 35 variants, 28 were classified as pathogenic or LP, indicating a diagnostic yield of 80%, while the remaining 7 (20%) were of unknown significance. Among the 29 patients with pathogenic or LP variants, 24 distinct variants were identified in 20 genes. The number of patients with variants, categorized by nosological groups, is shown in Table 1. Ten of these variants (detected in P6, P9, P11-15, P17, P19, P24, and P28) were novel, and ACMG classifications and in silico predictions of those variants were provided in Supplementary Table 2.

Table 1. Number of patients in groups of nosology.

Nosology group no	Nosology group name	Gene	Patient number	
			P/ LP variant	VUS
1	*FGFR3* chondrodysplasias	*FGFR3*	3	1
2	Type 2 collagen disorders	*COL2A1*	5	
3	Type 11 collagen disorders	*COL11A1*		1
4	Sulfation disorders	*PAPSS2*	1	
5	Dysplasias with multiple joint dislocations	*SLC10A7*	1	
7	Proteoglycan core proteins disorders	*HSPG2*	1	
9	Pseudoachondroplasia and the multiple epiphyseal dysplasias	*COL9A1*	1	
11	Metaphyseal dysplasias	*COL10A1*	1	
		EFL1	2	
15	Mesomelic and rhizo-mesomelic dysplasias	*ROR2*	1	
		SHOX		2
17	Acromelic dysplasias	*FBN1*	1	
		GNAS	1	2
21	Primordial dwarfism and slender bones group	*CCDC8*	1	
23	Chondrodysplasia punctata (CDP) group	*MGP*	1	
24	Osteopetrosis and related osteoclast disorders	*CLCN7*	1	
26	Osteogenesis Imperfecta and bone fragility group	*COL1A1*	1	
		SERPINF1	1	
		TMEM38B	1	
34	Syndromes featuring craniosynostosis	*FGFR2*	2	
		FGFR3	1	
		TWIST1	1	
		SMO	1	
38	Limb hypoplasia – reduction defects group	-	1	

P: Pathogenic, LP: Likely pathogenic, VUS: Variant of unknown significance.

Fifteen patients with these variants exhibited an autosomal dominant inheritance pattern, four of whom inherited the disease from an affected parent. All 13 patients with an autosomal recessive disease had consanguineous parents. Additionally, one patient was found to have a somatic mosaic variant in the *SMO* gene, consistent with Curry-Jones syndrome (CRJS). In a patient initially diagnosed with fibular aplasia, tibial campomelia, and oligosyndactyly (FATCO) syndrome, no variants were identified in genes associated with these clinical features. The *COL2A1* gene, which encodes the collagen type II alpha 1 chain, was the most commonly affected gene in this group. Five patients were assigned to cluster 2 (type II collagen) and five to cluster 34 (syndromes with craniosynostosis) based on nosological classification. Within the FGFR3 chondroplasia group, the second nosology category, one patient exhibited a variant of uncertain clinical significance, while three patients carried pathogenic variants associated with achondroplasia. Variants identified in the patients are listed in Table 2.

Table 2. Variants detected in the participants in the presence of zygosity, pathogenicity, associated disease and familial segregation information.

Patient	Method	Variant	Zygosity	Variant classification (ACMG criteria)	Diagnosis (#OMIM; inheritance pattern)	Familial segregation
P1	Sanger Sequencing	*FGFR3*:NM_000142.4:c.1138G>A (p.Gly380Arg)	Het	Pathogenic	Achondroplasia (100800), AD	Father: Het Mother: Wt
P2	Sanger Sequencing	*FGFR3*:NM_000142.4:c.1138G>A (p.Gly380Arg)	Het	Pathogenic	Achondroplasia (100800), AD	NA
P3	Sanger Sequencing	*FGFR3*:NM_000142.4:c.1138G>A (p.Gly380Arg)	Het	Pathogenic	Achondroplasia (100800), AD	NA
P4	WES	*COL2A1*:NM_001844.5:c.1510G>A (p.Gly504Ser)	Het	Pathogenic	*COL2A1*-associated skeletal dysplasia (120280), AD	Father: Het Mother: NA
P5	WES	*COL2A1*:NM_001844.5:c.2059G>A (p.Gly687Ser)	Het	LP	*COL2A1*-associated skeletal dysplasia (120280), AD	Father: Wt Mother: Wt
P6	WES	*COL2A1*:NM_001844.5:c.2095G>A (p.Gly699Ser)	Het	LP	*COL2A1*-associated skeletal dysplasia (120280), AD	Father: Wt Mother: Wt
P7	CES	*COL2A1*:NM_001844.5:c.1510G>A (p.Gly504Ser)	Het	Pathogenic	*COL2A1*-associated skeletal dysplasia (120280), AD	Father: NA Mother: NA
P8	CES	*COL2A1*:NM_001844.5:c.1699G>A (p.Gly567Ser)	Het	Pathogenic	*COL2A1*-associated skeletal dysplasia (120280), AD	Father: NA Mother: NA
P9	CES	*PAPSS2*:NM_001015880.1:c.520+2T>A	Hom	LP	Brachyolmia 4 with mild epiphyseal and metaphyseal changes (612847), AR	Father: NA Mother: NA
P10	WES	*SLC10A7*:NM_001029998.6:c.335G>A (p.Gly112Asp)	Hom	LP	Short stature, amelogenesis imperfecta, and skeletal dysplasia with scoliosis (618363), AR	Father: NA Mother: NA
P11	WES	*HSPG2*:NM_005529.7:c.4948T>G (p.Cys1650Gly)	Hom	LP	Schwartz-Jampel syndrome, type 1 (255800), AR	Father: NA Mother: NA
P12	WES	*COL9A1*:NM_001851.4:c.1852C>T (p.Arg618Ter)	Hom	Pathogenic	Stickler syndrome, type IV (614134), AR	Father: NA Mother: Het
P13	CES	*COL10A1*: NM_000493.4:c.1766T>C (p.Phe589Ser)	Het	LP	Metaphyseal chondrodysplasia, Schmid type (156500), AD	Father: NA Mother: NA
P14	WES	*EFL1*:NM_024580:c.277T>C (p.Ser93Pro)	Hom	LP	Shwachman-Diamond syndrome 2 (617941), AR	Father: Het Mother: Het
P15	Sanger Sequencing	*EFL1*:NM_024580:c.277T>C (p.Ser93Pro)	Hom	LP	Shwachman-Diamond syndrome 2 (617941), AR	Father: NA Mother: NA
P16	WES	*ROR2*:NM_004560.4:c.355C>T (p.Arg119Ter)	Hom	Pathogenic	Robinow syndrome (268310), AR	Father: NA Mother: NA
P17	CES	*FBN1*:NM_000138.4:c.5285G>A p.(Gly1762Asp)	Het	LP	*FBN1*-associated skeletal dysplasia (134797), AD	Father: Het Mother: NA Affected sibling: Het

Table 2. Continued

Patient	Method	Variant	Zygosity	Variant classification (ACMG criteria)	Diagnosis (#OMIM; inheritance pattern)	Familial segregation
P18	CES	*GNAS:*NM_000516.5:c.125G>T (p.Arg42Leu)	Het	LP	Pseudopseudohypoparathyroidism (612463), AD	Father: NA Mother: NA
P19	CES	*CCDC8*:NM_032040.5:c.84_85delTA (p.Lys29AlafsTer118)	Hom	LP	3-M syndrome 3 (614205), AR	Father: NA Mother: NA
P20	Gene Panel	*MGP:*NM_000900.5:c.43del (p.Val15Ter)	Hom	LP	Keutel syndrome (245150), AR	Father: NA Mother: NA
P21	Gene Panel	*CLCN7*:NM_001287.6:c.1576C>T (p.Arg526Trp)	Hom	LP	Osteopetrosis, autosomal recessive 4 (611490), AR	Father: NA Mother: NA
P22	Gene Panel	*COL1A2*:NM_000089.4:c.2521G>A (p.Gly841Ser)	Het	LP	*COL1A2*-associated osteogenesis imperfect (120160), AD	Father: NA Mother: NA
P23	Gene Panel	*SERPINF1*:NM_002615.7:c.271_279dup (p.Ala91_Ser93dup)	Hom	LP	Osteogenesis imperfecta, type VI (613982), AR	Father: NA Mother: NA
P24	WES	*TMEM38B*:NM_018112.3:c.240_247dup (p.Ile83ThrfsTer21)	Hom	Pathogenic	Osteogenesis imperfecta, type XIV (615066), AR	Father: Het Mother: Het
P25	CES	*FGFR2:*NM_000141.4:c.1021A>C (p.Thr341Pro)	Het	Pathogenic	Crouzon syndrome (123500), AD	Father: NA Mother: NA
P26	WES	*FGFR2*:NM_000141.4:c.983A>G (p.Tyr328Cys)	Het	Pathogenic	Crouzon syndrome (123500), AD	Father: NA Mother: NA Siblings: NA
P27	CES	*FGFR3*:NM_000142.5:c.749C>G (p.Pro250Arg)	Het	Pathogenic	Muenke syndrome (602849), AD	Father: NA Mother: NA
P28	WES	*TWIST1*:NM_000474.4:c.464A>C (p.Tyr155Ser)	Het	LP	Saethre-Chotzen syndrome with or without eyelid anomalies (101400), AD	Father: Het Mother: Wt Affected twin: Het
P29	Sanger Sequencing	*SMO:*NM_005631.5:c.1234C>T (p.Leu412Phe)	Het (somatic; skin biopsy)	Pathogenic	Curry-Jones syndrome, somatic mosaic (601707)	Father: NA Mother: NA
P30	WES	-			FATCO syndrome (246570)	
P31	CES	*FGFR3:*NM_000142.5:c.1748A>T (p.Lys583Met)	Het	VUS	-	Father: NA Mother: NA
P32	WES	*COL11A1*:NM_001854.4:c.206G>C (p.Gly69Ala)	Het	VUS	-	Father: NA Mother: NA
P33	CES	*SHOX:*NM_000451.3c.847del (p.Ala283ArgfsTer124?)	Het	VUS	-	Father: NA Mother: NA
P34	CES	*SHOX:*NM_000451.3c.847del (p.Ala283ArgfsTer124?)	Het	VUS	-	Father: NA Mother: NA
P35	CES	*GNAS:*NM_080425.3:c.2968-3C>G	Het	VUS	-	Father: Wt Mother: Het
P36	CES	*GNAS:*NM_080425.3:c.2968-3C>G	Het	VUS	-	Father: Wt Mother: Het

LP: Likely pathogenic, VUS: Variant of unknown significance, Wt: Wildtype, Het: Heterozygous, Hom: Homozygous, AD: Autosomal dominant, AR: Autosomal recessive, WES: Whole exome sequencing, CES: Clinical exome sequencing, NA: Not applicable, ACMG: American College of Medical Genetics.

DISCUSSION

Skeletal dysplasia encompasses a highly heterogeneous group of genetic disorders, and its differential diagnosis can be difficult based on radiographic and clinical evaluation. Therefore, molecular techniques are valuable for precise diagnosis, thereby influencing disease management (13). Although the genetic basis of skeletal dysplasia has been described in various populations (14-18) and even in the Turkish population (19,20), further studies are needed to identify novel associated genes and variants.

This study presents molecular genetic findings in 36 Turkish patients with skeletal dysplasia. Using various sequencing methods for genetic diagnosis, the researchers successfully identified all but one patient at the molecular and diagnostic levels. Including Sanger sequencing, the diagnostic success rate reaches 80%. The *COL2A1* gene was the most affected in this group. Ages at diagnosis ranged from 1 month to 69 years, showing that skeletal dysplasia can have a wide range of severity. Patients were grouped into nosological categories based on their specific or probable diagnoses. The largest groups were group 2 (type II collagen disorders, 5 patients) and group 34 (syndromes with craniosynostosis, 5 patients). Variants of unknown significance in the *FGFR3, COL11A1, SHOX*, and *GNAS* genes, potentially associated with clinical features, were identified in six patients.

Type II collagen disorders are characterized by skeletal dysplasia, ocular manifestations (such as cataracts, myopia, lens subluxation, vitreous abnormalities, and retinal detachment), hearing loss, and orofacial features. They cause a wide range of diseases, from fatal conditions in the perinatal period to mild symptoms in adulthood (21). This study included five patients with pathogenic or LP variants in the *COL2A1* gene. All patients carried missense variants (Gly504Ser, Gly687Ser, p.Gly567Ser, and Gly699Ser) that lead to the substitution of glycine, a neutral, non-polar amino acid that is small enough to occupy the center of the collagen triple helix, with serine, a neutral, polar amino acid. Due to the unique structural role of glycine residues in the collagen triple helix, missense substitutions have a greater impact than truncating variants, making them the most common pathogenic variants (22). Aside from Gly699Ser, the other variants had been identified previously. The Gly504Ser variant has frequently been associated with the spondyloepiphyseal dysplasia congenita (SEDC) phenotype (23,24). In a cohort study of 93 patients with SEDC or related phenotypes, glycine substitutions in the triple-helical domain were identified in 68 (73%) patients. Additionally, in 28 of these patients, glycine was replaced by serine (25).

In group 34 of the nosological classification, which includes syndromes with craniosynostosis, pathogenic variants were identified in FGFR2 (two patients) and in FGFR3, TWIST1, and SMO (one patient each). A somatic mosaic c.1234C>T (p.Leu412Phe) variant in the SMO gene was identified in P29, who had macrocephaly, facial and cranial asymmetry, frontal bossing, microphthalmia of the left eye, low-set ears, and bilateral polysyndactyly (Figures 1a and 1b). This variant causes CRJS, which is a skeletal dysplasia characterized by polysyndactyly, craniosynostosis, microphthalmia, and patchy skin lesions. Within this group, a small number of cases of CRJS have been reported in the literature (26). While most findings in P29

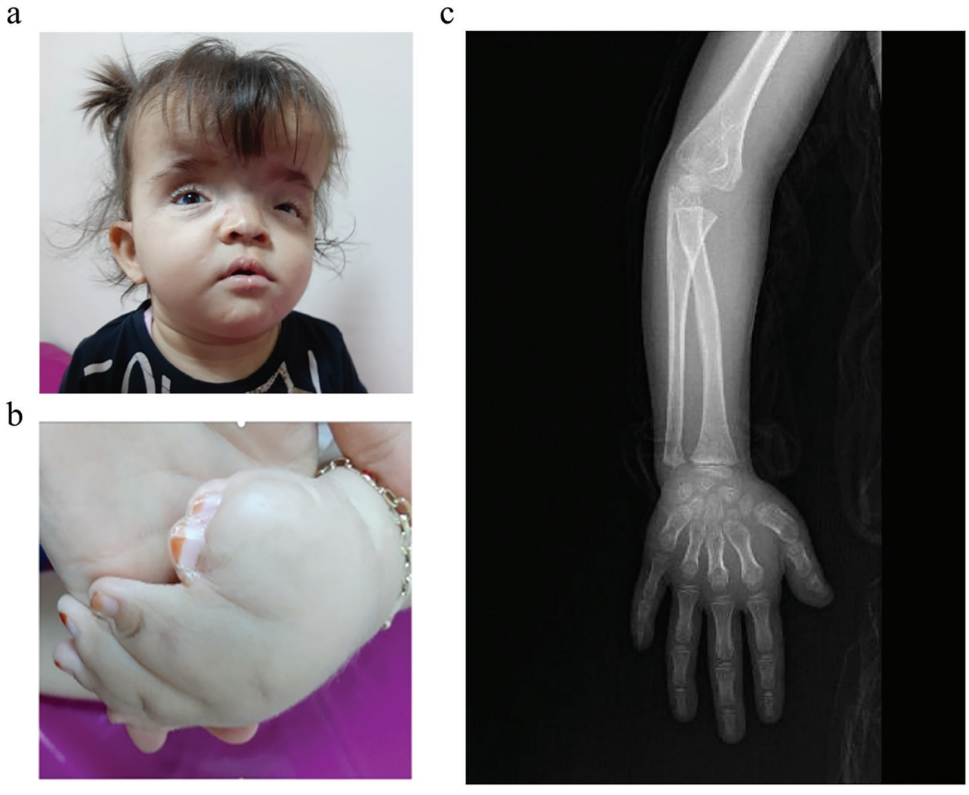

Figure 1. Clinical images of the selected patients. a) Dysmorphic facial features of the P29; macrocephaly, facial and cranial asymmetry, frontal bossing, microphthalmia of the left eye, low-set ears. b) Polysyndactyly of the right hand of the P29. c) X-ray image of P9's right hand showing short metacarpals (2^{nd}–5^{th}), with the 5^{th} metacarpal being the most prominent.

match those of other patients described in the literature, this patient also exhibited bilateral sensorineural hearing loss.

Patient 9, with short stature (-3.27 SD), short neck, pectus carinatum, short metacarpals of the right hand (Figure 1c), and mild epiphyseal and vertebral changes, carried a homozygous LP variant in the 3-*prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 (PAPSS2)* gene, classified as group 4 in the nosology. *PAPSS2* has been associated with brachyolmia type 4, characterized by a short trunk, mild shortening of the metacarpal bones, mild epiphyseal and metaphyseal changes in the tubular bones, a short femoral neck, and vertebral changes (rectangular vertebral bodies with irregular end plates and narrow intervertebral discs; OMIM #612847). *PAPSS2*-related brachyolmia results from a loss-of-function mechanism (27). The c.520+2T>A variant detected at P9 is located in intron 4. This variant is predicted to cause nonsense-mediated mRNA decay. Alternatively, if exon skipping occurs, exon 5, which constitutes part of the adenylyl-sulfate kinase domain, is also predicted to be affected. This variant has been reported as homozygous in two patients with disproportionate short stature and platyspondyly and as compound heterozygous in another case (27). Compared with other patients reported in the literature, whether or not they had the same variant, P9 lacked a short trunk, scoliosis, and platyspondyly. Since P9 is only 4.5 years old, further findings may emerge. Variable expressivity should also be considered. A summary of clinical findings for patients with biallelic *PAPSS2* variants is available in Supplementary Table 3.

In P10, a homozygous LP variant was found in the *SLC10A7* gene; this variant is linked to a group of dysplasias involving multiple joint dislocations. The gene is associated with autosomal recessive short stature, amelogenesis imperfecta, and skeletal dysplasia with scoliosis (OMIM #611459). The c.335G>A (p.Gly112Asp) variant in transmembrane domain 4, present in P10, was previously identified as part of compound heterozygosity with a splicing variant in siblings (28). Another report described this variant paired with p.Gly130Arg, also in exon 4 of the same gene (29). As detailed in Supplementary Table 4., all patients with biallelic pathogenic variants in *SLC10A7* exhibited amelogenesis imperfecta, with nearly all showing kyphoscoliosis. Unlike other patients, P10 did not have intellectual disability or hearing loss, but had pectus deformity and a narrow thorax—features absent in other patients. These differences may result from compound heterozygosity. Additionally, azoospermia was observed in P10, representing a novel finding. His hormonal profile and scrotal ultrasound results were normal. A previous report described azoospermia in an SLC10A7-associated case that also exhibited a structural chromosomal abnormality ([nv ins(2;4)] involving SLC10A7 at the breakpoint (30). However, further studies are needed to elucidate the relationship between this finding and *SLC10A7*.

P16, carrying an LP *FBN1* variant, was classified within the 17th nosology group, known as acromelic dysplasias. This group includes *FBN1*-associated geleophysic dysplasia (GD), acromicric dysplasia, and Weill-Marchesani syndrome. The c.5285G>A p.(Gly1762Asp) variant detected in P16 has not been previously reported, although similar variants, such as Gly1762Val and Gly1762Ser, have been linked to GD (31,32). Considering the features observed in P16—disproportionate short stature, short limbs, and brachydactyly—the diagnosis was deemed consistent with GD.

P30, diagnosed with FATCO syndrome, was categorized in group 38 (limb hypoplasia–reduction defects). FATCO syndrome involves fibular aplasia, tibial campomelia, and oligosyndactyly, although its genetic basis remains unknown. The WES test for P30 did not detect any variants linked to the observed features.

This study was limited because some patients had limited skeletal imaging or limited physical exam findings, which hindered full identification of disease-related features. Furthermore, in the group with variants of uncertain clinical significance, there remains a possibility of a different genetic cause—either not detected by the CES analysis or attributable to mechanisms outside the scope of CES/WES methods.

CONCLUSION

In genetic disorders, such as skeletal dysplasias, where molecular diagnostics may not always specify subtypes, discovering new variants and characterizing clinical features that resemble or differ from those of known variants are essential for accurate diagnosis and treatment. We believe that this study will enhance the understanding of genotype-phenotype relationships by presenting clinical data from patients with genetic skeletal diseases.

Ethics

Ethics Committee Approval: The present study was approved by the Ethics Committee of Gazi University (decision number: 403, date: 30.05.2022).

Informed Consent: It was obtained

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Footnotes

Authorship Contributions

Surgical and Medical Practices: E.U., F.P., A.B., H.H.K., Y.B., G.K., Concept: E.U., F.P., A.B., G.K., Design: E.U., F.P., A.B., G.K., Data Collection or Processing: E.U., F.P., A.B., H.H.K., Y.B., G.K., Analysis or Interpretation: E.U., F.P., A.B., H.H.K., Y.B., G.K., Literature Search: E.U., H.H.K., Y.B., G.K., Writing: E.U., F.P., A.B., H.H.K., Y.B., G.K.

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Supplementary Tables 1–4: https://d2v96fxpocvxx.cloudfront.net/580eb5e7-1480-44a6-9404-b8b7446acbcb/content-images/4cd53ab1-61c1-4f0e-bd54-0d67d3a78b16.pdf