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Diagnostic Challenge of Phenotypic Variability in *COL2A1*-related Disorders: Four Novel Variants That Expand the Clinical Spectrum

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What is already known on this topic?

Type 2 collagenopathies are caused by heterozygous mutations in the *COL2A1* gene, leading to a broad spectrum of clinical phenotypes ranging from mild to severe skeletal dysplasias. These disorders are characterized by diverse clinical presentation, including short-trunk dwarfism, joint pain, ocular complications, and hearing loss. Despite the variability in clinical manifestations, accurate diagnosis and classification of these disorders are essential for effective genetic counseling and management.

What this study adds?

This study expands the clinical and molecular spectrum of *COL2A1*-related disorders by identifying four novel variants (c.1023 + 2T > C, p.Gly465Asp, p.Gly855Asp, p.Gly669Ala) in patients with kniest dysplasia, spondyloepiphyseal dysplasia congenita, and spondyloepimetaphyseal dysplasia Strudwick type. The findings highlight the phenotypic variability and diagnostic challenges associated with type 2 collagenopathies, highlighting the need for comprehensive genetic and radiological assessments for accurate diagnosis.

Abstract

Objective: Heterozygous *COL2A1* gene mutations are associated with type 2 collagenopathies, characterized by a wide, diverse, and overlapping clinical spectrum in related diseases. Our goal is to describe the clinical, radiological, and molecular findings of patients with *COL2A1*-related dysplasia and investigate the phenotype-genotype correlation. We also highlight the challenge of categorizing *COL2A1*-related diseases with similar clinical and radiological phenotypes.

Methods: Six patients from five unrelated families presented with disproportionate short stature.delayed motor milestones, waddling gait, normal intelligence, and similar radiological features, including delayed epiphyseal ossification, epimetaphyseal changes, scoliosis, lordosis, and platyspondyly. All underwent whole exome sequencing. Demographic, clinical, laboratory, and radiological data were retrospectively obtained from hospital records. Segregation analysis was conducted using Sanger sequencing in all patients.

Results: Based on clinical, radiological, and molecular results, the six patients were categorized into kniest dysplasia, spondyloepiphyseal dysplasia congenita, and spondyloepimetaphyseal dysplasia Strudwick type. Four novel variants (c.1023 + 2T > C, p.Gly465Asp, p.Gly855Asp, p.Gly669Ala) were identified in the *COL2A1* gene.

Conclusion: Accurate classification of type 2 collagenopathies is vital to provide appropriate genetic counseling. Predicting extraskeletal manifestations and reducing morbidity through early diagnosis and treatment will significantly improve the quality of life for patients.

Keywords: *COL2A1* gene, *COL2A1*-related disorders, short-trunk dwarfism, type 2 collagenopathies, whole exome sequencing

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Introduction

The most prevalent proteins in the human body, collagens, are involved in the structure and mechanical qualities of tissues (1). One of the fibrillar collagens, type 2 collagen, is mainly present in cartilage, and partially in the vitreous humor, inner ear, and nucleus pulposus (2). It is the main protein of endochondral bone development and growth. The collagen type 2 alpha-1 gene (*COL2A1*, MIM *120140) is located on the long arm of chromosome 12 at band 13.11 and is 31.5 kb in size. According to the common transcript (NM_001844.4), it is a gene with 54 exons that encodes the type 2 pro-collagen alpha 1 protein. This protein forms a triple helical structure and assembles into a pro-collagen homotrimer structure consisting of approximately 300 Glycine-X-Y amino acid repeats. The type 2 pro-collagen molecule has non-helical N- and C-terminal propeptides that do not contain Gly-X-Y repeats. After being secreted into the extracellular matrix, type 2 pro-collagen undergoes cleavage of N- and C-terminal propeptides to form mature collagen (3).

Heterozygous *COL2A1* gene mutations are referred to as type 2 collagenopathies, and the associated diseases exhibit a broad, heterogeneous, and overlapping clinical spectrum (Table 1). The disease spectrum ranges from only osteoarthritis with normal stature or ocular complications and hearing loss to severe micromelia, dwarfism, and perinatal lethality. Achondrogenesis type 2 (ACG2) or hypochondrogenesis (HCG) and platyspondylic skeletal dysplasia Torrance type are perinatal lethal forms; kniest dysplasia (KD), spondyloepiphyseal dysplasia congenita (SEDC), spondyloepimetaphyseal dysplasia (SEMD) Strudwick type, spondyloepiphyseal dysplasia Stanescu type and spondyloperipheral dysplasia are moderate forms; epiphyseal dysplasia multiple with myopia and deafness, vitreoretinopathy with phalangeal epiphyseal dysplasia, avascular necrosis of the femoral head, Czech dysplasia, Legg-Calve-Perthes disease, osteoarthritis with mild chondrodysplasia, Stickler syndrome type 1 (STL1), and non-syndromic ocular STL1 are mild forms (4). While the overall prevalence remains unknown, the estimated incidence worldwide ranges from 20.4 to 35.9/100,000 across various locations and populations (5). Due to *COL2A1* mutations leading to different phenotypes, even within the same family, clinical variability is observed.

Table 1. Diseases associated with *COL2A1* mutation

Phenotype	MIM number
Epiphyseal dysplasia, multiple, with myopia and deafness	132450
Vitreoretinopathy with phalangeal epiphyseal dysplasia	619248
Achondrogenesis, type 2 or hypochondrogenesis	200610
Avascular necrosis of the femoral head	608805
Czech dysplasia	609162
Kniest dysplasia	156550
Legg-Calve-Perthes disease	150600
Osteoarthritis with mild chondrodysplasia	604864
Platyspondylic skeletal dysplasia, Torrance type	151210
Spondyloepiphyseal dysplasia congenita	183900
Spondyloepimetaphyseal dysplasia, Strudwick type	184250
Spondyloepiphyseal dysplasia, Stanescu type	616583
Spondyloperipheral dysplasia	271700
Stickler syndrome, type 1	108300
Stickler syndrome, type 1, non-syndromic ocular	609508

MIM: Mendelian inheritance in man

In general, when type 2 collagenopathies are mentioned, the first things that come to mind are short trunk dwarfism, eye involvement (myopia and vitreoretinal detachment), hearing loss, and joint pain. Cleft palate, midface hypoplasia, and micrognathia may also be considered dysmorphic facial features. Radiographic manifestations include platyspondyly, irregular vertebral endplates, kyphosis, lordosis, delayed epiphyseal ossification, and epimetaphyseal changes.

Short stature is one of the problems that pediatric endocrinologists and geneticists spend a significant amount of time on. A considerable proportion of these patients are composed of skeletal dysplasias, and genetic counseling is important in this respect. Here, we report the clinical, radiological, and molecular genetic features of six patients from five different families and report four novel variants in the *COL2A1* gene, expanding the molecular spectrum of the diseases with a clinical diagnostic challenge.

Methods

Five of the six patients were of Turkish descent, with one being an Uzbek refugee. All six exhibited short-trunk dwarfism, a waddling gait, normal intelligence, and similar radiological findings. They underwent whole exome sequencing, and an expert clinical geneticist examined all patients. Demographic, clinical, laboratory, and radiological data were retrospectively collected from hospital records.

Genomic DNA was extracted from peripheral blood using instruments and a DNA blood 520 µL kit (Xiamen Zeesan Biotech, Fujian, China) following standard protocols. Whole exome capture and sequencing were performed using the Twist Human Core Exome V2 Kit (South San Francisco,

CA, USA). Libraries were sequenced on a NovaSeq 6000 system (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. Variant calls from FASTQ files were generated with the Sophia DDM version 5.10.50.1 (Lausanne, Switzerland) bioinformatics platform, which was also used for variant annotation and analysis.

Initially, variants with a minor allele frequency greater than 0.01 were excluded using data from the 1000 Genomes Project and gnomAD databases. Genes associated with the patients' phenotype, especially those related to skeletal dysplasia, were prioritized. Candidate variants were assessed using the ClinVar, LOVD, and HGMD databases along with relevant publications. The pathogenicity of identified variants was evaluated using several *in silico* prediction tools, including DANN, DEOGEN2, EIGEN, MutationTaster, Polyphen-2, and SIFT. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (6). Segregation analysis of all variants in the families was conducted using Sanger sequencing.

Written informed consent for genetic testing and publication of clinical findings, patient photographs, and molecular results was obtained from each patient or their parents. The study adhered to the Declaration of Helsinki and was approved by the Ethical Committee of University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, (approval number: B.10.1.TKH.4.34.H.GP.0.01/293, date: 05.09.2024).

Statistical Analysis

Descriptive statistics of the clinical and laboratory findings are expressed as mean ± standard deviation, numbers, or percentages. Comparative statistics were not performed because the number of patients was limited and not homogeneously distributed.

Results

Clinical Features

Patient 1: A boy was referred at four months of age due to dysmorphic facial features. He was born to non-consanguineous parents at 40 weeks of gestation. His birth weight was 3500 g (58th percentile), length was 51 cm (67th percentile), and head circumference was 38.5 cm [+2.5 standard deviation score (SDS)]. His neuromotor development was normal except for delayed walking, which occurred at 18 months. At his last physical examination at three years of age, his weight was 13.5 kg (23rd percentile), height was 90 cm (4th percentile), and head circumference was 52.5 cm (94th percentile). Notable dysmorphic features included relative macrocephaly, midface hypoplasia, hypertelorism, a depressed nasal bridge, a fleshy and upturned nose with anteverted nares, a bifid uvula, and micrognathia. In addition, he exhibited short trunk dwarfism and lordosis. He had a waddling gait. Ophthalmological and hearing examinations were normal, and other system examinations revealed no abnormalities.

Patient 2: A 3-year-old girl with short stature was referred for evaluation of possible skeletal dysplasia. She is the third child of a consanguineous, healthy Uzbek couple with no notable family history. She was born full-term with a birth weight of 3200 g (40th percentile) and a length of 43 cm (-3 SDS) with an unknown head circumference. Short-trunk dwarfism was noted at birth. She required a 20-day stay in the neonatal intensive care unit for respiratory distress. There was a delay in all motor milestones, with walking achieved at 2.5 years. At six years old, her weight was 14 kg (-2.9 SDS), height 88 cm (-5.9 SDS), and head circumference 52 cm (72nd percentile). Her facial features were normal except for a depressed nasal bridge and hypertelorism. She had limited and painful extension in her knee and elbow joints, genu valgum, and a waddling gait. She experienced hearing loss and myopia. Spinal magnetic resonance imaging showed odontoid hypoplasia, but her neurological examination was normal.

Patient 3: The boy, the fourth child of healthy unrelated parents, was noted to have short limbs on prenatal ultrasound. Postnatal history included a three-month stay in the neonatal intensive care unit for respiratory distress and surgeries for inguinal hernia and cleft palate. Motor milestones were delayed. At 12 years old, his weight was 27 kg (-2.4 SDS), height 90 cm (-8.3 SDS), and head circumference 55 cm (0.2 SDS). He had no facial dysmorphism but exhibited pectus carinatum, kyphosis, and lordosis. He had hearing loss but his eye examination was normal. Cervical computed tomography revealed atlantoaxial dislocation and cord compression.

Patient 4: The girl, the second child of healthy unrelated parents, was born at 38 weeks of gestation. Short limbs were noted on prenatal ultrasound. Her birth weight was 3800 g (87th percentile), height 46 cm (5th percentile), and head circumference 35 cm (64th percentile). Postnatally, she spent 10 days in the neonatal intensive care unit for respiratory distress and had congenital hypotonia and hip dysplasia. Motor development was delayed, with head control at one year, sitting without support at 14 months, and walking at two years. At 4.5 years old, her weight was 10.5 kg (-4.5 SDS), height 75 cm (-7.8 SDS), and head circumference 52.5 cm (86th percentile). Facial features were normal except for a prominent forehead and micrognathia. Short-trunk

dwarfism, pectus excavatum, and genu valgum were noted. She complained of pain in large joints with no hearing or visual impairments.

Patient 5: The boy, noted to have short limbs on prenatal follow-up, was born at 33 weeks of gestation. His birth weight was 2080 g (50-90th percentile), height 38 cm (< 3rd percentile), with an unknown head circumference. He spent three months in the neonatal intensive care unit for respiratory distress. Neuromotor development was normal except for walking at 18 months. At 5 years and 10 months, his weight was 16.4 kg (-2 SDS), height 90 cm (-5.3 SDS), and head circumference 50.5 cm (18th percentile). Facial features were normal. Physical examination revealed short-trunk dwarfism and a barrel chest. He exhibited a waddling gait after excessive walking. Eye and hearing examinations were normal. His mother also had marked short stature.

Patient 6: The affected mother of patient 5. Prenatal and natal history are unclear, and her birth measurements are unknown. She had surgery for hip dysplasia in childhood. On physical examination, her weight was 43 kg (-2.8 SDS), height 123 cm (-6.6 SDS), and head circumference 54 cm (-2 SDS). Facial features were normal. She had short-trunk dwarfism and scoliosis. Ophthalmological and hearing examinations were normal.

Common Findings

In all six patients, metabolic screening (calcium, phosphorus, alkaline phosphatase, parathyroid hormone, 25-hydroxy vitamin D, and thyroid hormone) and biochemical tests for liver and kidney functions were normal. Abdominal ultrasonography and echocardiography were normal. All patients had normal intelligence. Clinical and radiological characteristics of the patients are provided in Table 2, with photographs in Figure 1.

Radiological Findings

All of the patients' radiographic findings exhibit a remarkable degree of resemblance. Short long bones and the absence of ossification of capital femoral epiphyses were observed in all patients. All patients had insufficient pubic ossification except for patient 5 and patient 6. Platyspondyly with irregular vertebral end-plates were detected in patients 1, 3, 4, and 6. Irregular and mildly flared metaphyses were seen in patients 1, 2, 3, and 4. Various degrees of lordosis were present in all patients except for patient 4. The radiological images of the patients are described in detail in Figure 2.

COL2A1 Variants

Whole exome sequencing was performed, and five different heterozygous *COL2A1* variants were detected in the six patients from five families (Table 2). These variants were classified according to ACMG criteria. Two of the patients were a mother and her son. Four variants were not previously reported in the literature. One of the patients had a splicing variant, while the others had missense variants. The splicing variant was c.1023+2T>C (PVS1, PM2) in patient 1. In patient 2, molecular analysis identified the c.905C>T (p.Ala302Val) (PM2, PP5) variant in exon 14. This missense variant has been previously reported. The c.1394G>A (p.Gly465Asp) (PM2, PM5, PP3) variant in exon 22 was a novel variant and was detected in patient 3. The other novel variant was c.2564G>A (p.Gly855Asp) (PM2, PM5, PP3) in exon 39 in patient 4. patient 5 and her mother, patient 6, were found to have the c.2006G>C (p.Gly669Ala) (PM2, PP3) missense variant in exon 31, which has not been reported in public databases or literature. Segregation analysis revealed that patients 1-4 and 6 inherited the variants *de novo*, while patient 5 had maternal inheritance from patient 6, his mother. All variants are shown in Figure 3. The pedigrees of the patients with novel *COL2A1* variant are shown in Figure 4. In addition, biomolecular modellings of the *COL2A1* novel missense variants are shown in Figure 5.

Discussion

The disorders associated with *COL2A1* exhibit a broad range of phenotypes. Radiological signs and clinical findings of type 2 collagenopathies are very similar, making it quite difficult to diagnose. Phenotypic variability is most likely a result of environmental influences and variations in disease-altering genes and/or regulatory components. In the present case series, six patients were classified into KD (n=3; P1, P2, P4), SEDC (n=2; P5 and P6), and SEMD Strudwick type (n=1; P3) based on clinical, radiological, and genetic results. Five variants were identified, four of which were novel. We believe that our clinical, radiological, and genetic findings may be helpful in evaluating diseases associated with *COL2A1*.

According to the Human Gene Mutation Database (HGMD Professional 2024.1; HGMD June 2024), there are 759 pathogenic and likely pathogenic *COL2A1* variants. Among these, 423 are missense/nonsense, 137 are small deletions, 129 are splicing, 40 are small insertions, 16 are small indels, 10 are large deletions, and the others are large insertions and complex rearrangements. The majority of *COL2A1* gene variations are missense variants, and the substitution in glycine residues is the most prevalent form (7). Moreover, most of the variations are located in the triple helix domain of the protein. One of the patients in our study had a splicing variant, while the remaining patients had missense

Table 2. Clinical summary of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
***COL2A1* heterozygous mutation (NM_001844.4)**	c.1023 + 2T > C	c.905C > T:p. Ala302Val	c.1394G > A:p. Gly465Asp	c.2564G > A:p. Gly855Asp	c.2006G > C:p. Gly669Ala	c.2006G > C:p. Gly669Ala
Mutation type	Splicing	Missense	Missense	Missense	Missense	Missense
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	Maternal	*De novo*
Novel variant	+	-	+	+	+	+
Gender	Male	Female	Male	Female	Male	Female
Age of diagnosis (years)	0.42	3	7.5	1.5	1 y	28
Age at last admission (years)	3	6	12	4.5	5 y 10 mo 5.83	32
Short-trunk dwarfism, identifiable at birth	-	+	+	+	+	?
Height SDS	-2	-5.9	-8.3	-7.8	-5.3	-6.8
Weight SDS	-0.9	-2.9	-2.4	-4.5	-2	-2.8
Head circumference SDS	1.5	0.5	0.2	1.4	-0.9	-2
Congenital hypotonia	-	+	+	+	-	+
Flat midface	+	+	-	-	-	-
Round face	+	+	-	-	-	-
Malar hypolasia	-	-	-	-	-	-
Short neck	+	+	+	+	+	+
Cleft palate	-	-	+	-	-	-
Hearing loss	-	+	+	-	-	-
Myopia	-	+	-	-	-	-
Retinal detachment	-	-	-	-	-	-
Pectus excavatum	-	-	-	+	-	-
Pectus carinatum	-	-	+	-	-	-
Barrel chest	-	-	-	-	+	-
Inguinal hernias	-	-	+	-	-	-
Atlanto-axial instability	-	+	+	-	-	-
Odontoid hypoplasia	-	+	+	-	-	-
Platyspondyly	+	-	+	+	-	+
Irregular vertebral endplates	+	+	+	+	-	+
Coronal vertebral clefts	-	-	+	-	-	-
Kyphosis	-	-	+	-	-	-
Scoliosis	-	+	+	-	-	+
Lordosis	+	+	+	+	+	+
Dislocation of hip, congenital	-	?	-	+	-	+
Coxa vara	-	-	-	-	-	-
Coxa valga	+	-	-	-	-	-
Flexion contractures, especially at hip and knee	-	+	-	-	-	+
Absent pubic ossification, infancy	+	+	+	+	+	?
Delayed epiphyseal ossification, childhood	+	+	+	+	+	+
Enlarged epiphyses	+	-	-	-	-	-
Irregular epiphyses	+	+	+	+	-	+
Metaphyseal irregularity	+	+	+	+	-	-
Osteosclerosis/osteopenia zones	-	-	+	-	-	-
Short, dumbbell appearance of long bones	+	-	-	+	-	-
Limited joint mobility	-	+	-	-	-	+
Painful joints	-	+	+	+	-	-
Enlarged joints	+	+	-	-	-	-
Delayed motor milestones	+	+	+	+	+	+
Abnormal gait	+	+	+	+	+	-
Normal intelligence	+	+	+	+	+	+

SDS: standard deviation score

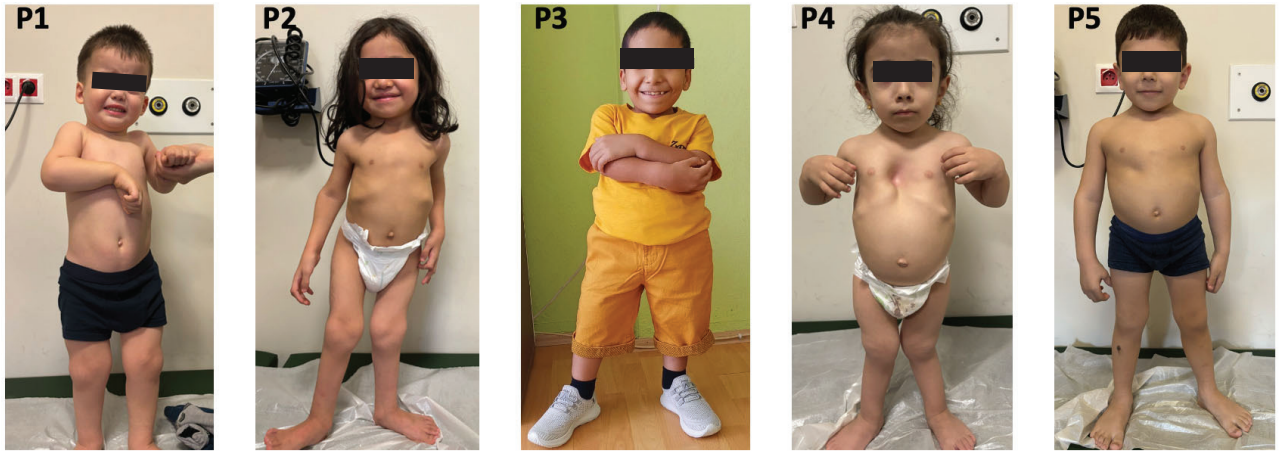

Figure 1. Photographs of the patients 1-5. All patients have short trunk dwarfism. **P1** (3 years old): Round face, midface hypoplasia, depressed nasal bridge, micrognathia, enlarged knee and elbow joints. **P2** (6 years old): Round face, depressed nasal bridge, enlarged knee and elbow joints, genu valgum deformity. **P3** (12 years old): Enlarged wrists. **P4** (4,5 years old): Micro/retrognathia, pectus excavatum and genu valgum deformity. **P5** (5 years 10 months old): Barrel chest

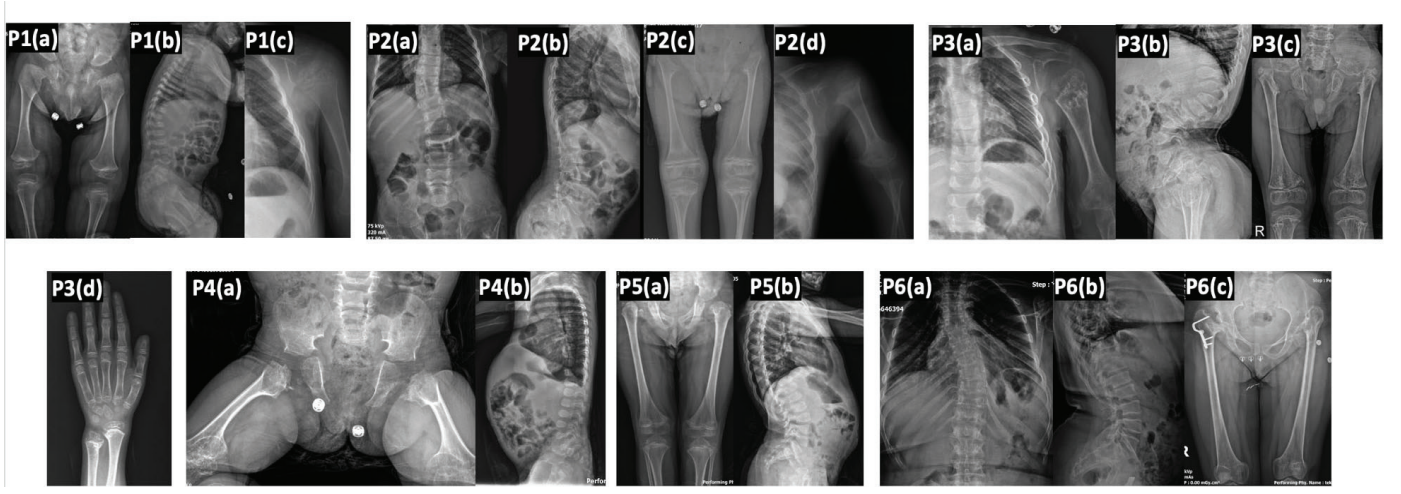

Figure 2. X-rays of all the patients. All tubular long bones are short. **P1(a)** (14 months old**):** Insufficient pubic ossification, absence of ossification of capital femoral epiphyses, very broad femoral necks, irregular femur distal metaphyses and epiphyses, large proximal tibiae epiphyses, short-dumbbell shaped femur, coxa valga. **P1(b)** (2 months): Platyspondyly with irregular vertebral endplates, mild lordosis. **P1(c)** (3 years old): Expanded and irregular epiphysis with mildly broad diaphysis. **P2(a)** (5 years old): Thoracolombar scoliosis. **P2(b):** Irregular vertebral endplates, lordosis. **P2(c):** Small with horizontal inferior margins of the ilia, insufficient pubic ossification, absence of capital femoral epiphyses, short and broad femoral necks, coxa valga, irregular and mild flared metaphyses, irregular epiphyseal margins especially on the left side. **P2(d)** (2 years old): Dumbbell shaped humerus. **P3(a)** (10 years old): Thoracal scoliosis and enchondroma-like appearance in the proximal metaphysis of the humerus. **P3(b):** Platyspondyly with irregular vertebral endplates, posterior wedging of lower lomber vertebra, kyphosis, severe lordosis. **P3(c):** Short ilium, horizontal acetabula, narrow and shallow sciatic notch, lack of ossification of the os pubis, absence of capital femoral epiphyses and necks, irregular metaphyses and epiphyses. **P3(d):** Epiphyseal and metaphyseal irregularity. Osteosclerosis and osteopenia zones at distal radius and ulna metaphyses. Carpal bone ossification is delayed. **P4(a)** (4 years old): Horizontal acetabula, absence of pubic ossification, absence of capital femoral epiphysis on the left side, very small capital femoral epiphysis on the right side, short-dumbbell shaped femur, irregular and enlarged metaphyses. **P4(b)** (1 year old): Platyspondyly with irregular vertebral endplates (pear-shaped appearance). **P5(a)** (5 years 10 months old): Absence of capital femoral epiphyses, multicentric ossification of right femoral neck, small and irregular femoral necks, coxa valga, small proximal tibia epiphyses. **P5(b):** Flattaned with pear-shaped vertebrae and lordosis. **P6(a)** (mother of Patient 5): Thoracal scoliosis. **P6(b):** Platyspondyly with irregular vertebral endplates, posterior wedging of lower lomber vertebra, lordosis. **P6(c):** Irregular margins of ilia, shallow acetabular fossa, irregular ossified (deformed) femoral necks, absence of femoral heads

variants. Among the patients with missense variants, only one exhibited an alanine-valine substitution, while all others had glycine substitutions.

There are two main mechanisms through which pathogenic molecular variants in the *COL2A1* gene might cause diseases: haploinsufficiency and dominant-negative effects (4). Missense variations resulting in the substitution of the glycine residue within the Gly-X-Y triplet exhibit a dominant-negative effect, causing the disturbance of the helical structure and function of type 2 collagen (8).

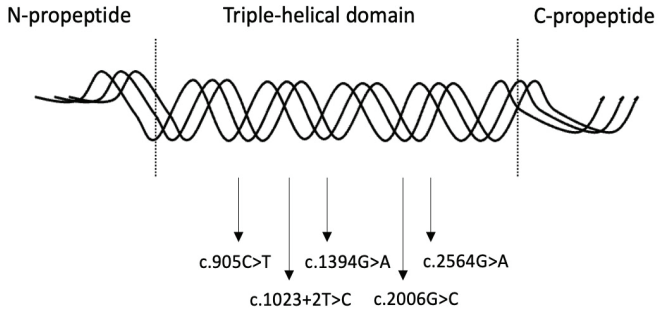

Figure 3. Schematic representation of the variants in our study based on domains of the type 2 pro-collagen trimer

Haploinsufficiency is caused by pathogenic mutations that result in the premature termination of protein synthesis. The dominant-negative effect causes more severe type 2 collagenopathies, whereas haploinsufficiency leads to a mild phenotype. Furthermore, substituting amino acids other than glycine through missense mutations typically results in a less severe phenotype due to the destabilization of the protein (9).

There is a lack of data regarding the genotype-phenotype correlation in type 2 collagenopathies. However, Zhang et al. (10) reported some potential genotype-phenotype correlations, based on the location of variants. Variants in the N-propeptide region, particularly in exon 2, result in mild symptoms, such as in STL1. In contrast, variants in the C-propeptide region give rise to severe and often lethal phenotypes. Replacing glycine with serine results in phenotypes that range in severity from mild to severe. Conversely, substituting glycine with a non-serine amino acid leads to more severe phenotypes, such as HCG or SEDC, often accompanied by severe coxa vara (11). The non-glycine missense mutations primarily involve the substitution of arginine with cysteine, resulting in mild phenotypes characterized by either normal or short stature (12). Small deletion variants, excluding frameshift variants caused by nucleotide deletions, result in severe or fatal diseases.

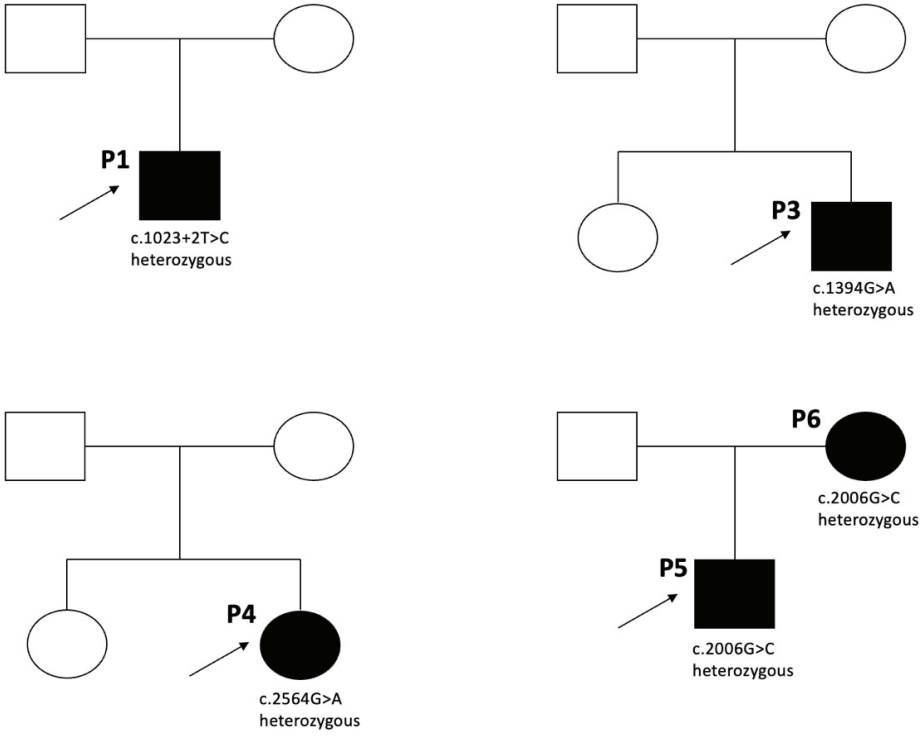

Figure 4. Pedigrees of the patients with novel *COL2A1* variants

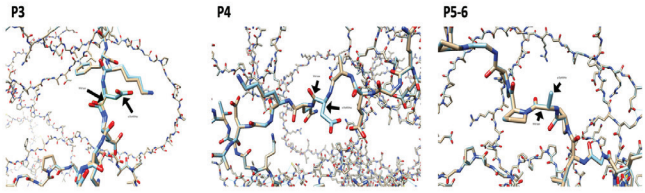

Figure 5. Biomolecular modeling of the collagen alpha-1(2) chain (AF-P02458-F1-v4) with three novel variants: p.(Gly465Asp), p(Gly669Ala), and p.(Gly885Asp)

KD is characterized by short-trunk dwarfism, flat midface, prominent eyes, cleft palate, myopia, retinal detachment, cataract, hearing loss, umbilical/inguinal hernia, enlarged painful joints, and limited joint mobility. Radiological findings of KD are kyphoscoliosis, odontoid hypoplasia, atlanto-axial instability, coronal vertebral clefts, platyspondyly, short and broad ilia, delayed epiphyseal ossification, epimetaphyseal irregularity, and short-dumbbell shaped long bones. The ossification of the proximal femoral epiphyses is significantly delayed and may not occur until puberty in severe involvement (13). Since the dominant features in patients 1, 2, and 4 were delayed epiphyseal ossification, metaphyseal and epiphyseal changes, and dumbbell-like long bones, a diagnosis of KD was made. The features of SEDC are very similar to KD. However, micro/retrognathia, pear-shaped vertebral bodies, almost unaffected metaphyses, and the absence of dumbbell-like long bones may distinguish it from KD. Patients 5 and 6 were diagnosed with SEDC because they exhibited epiphyseal involvement and preserved metaphyses in X-rays. In patient 3, in addition to epiphyseal delay and metaphyseal changes, the presence of metaphyseal sclerosis in the distal radius and ulna and an enchondroma-like appearance in the proximal metaphysis of the humerus led to the diagnosis of SEMD Strudwick type. SEMD Strudwick type can be considered a form of SEDC because the radiological appearance is very similar to SEDC in the neonatal period. However, metaphyseal changes begin to occur during childhood. In addition to enlarged, irregular, and mottled metaphyses, an enchondroma-like appearance and zones of osteosclerosis and osteopenia at the metaphyses are also notable (13). As mentioned before, it may be difficult to conclusively determine diagnoses because clinical and radiological findings are closely intertwined.

In the present study, one splicing variant (c.1023 + 2T > C) and two missense variants (c.905C > T p.Ala302Val; c.2564G > A p.Gly855Asp) were associated with KD. Zhang et al. (10) identified a hotspot variant c.905C > T in KD. Chen et al. (14) previously conducted a functional analysis on this variant, demonstrating its impact on the splicing process by causing the skipping of 21 nucleotides. The splicing variant c.1023 + 2T > C has not been reported previously. The c.1023 + 2T > C variant was analyzed using the SpliceAI tool. This variant is located in the canonical splice donor site region of exon 16 of this 54-exon gene. The variant is predicted to affect mRNA splicing and lead to out-frame intron retension. However, this mechanism must be supported by functional studies. It is worth mentioning that splicing variants in other genes have precise explanations, such as alterations in the length of exons or the skipping of exons resulting in premature stop codons. The distinctive architecture of collagen proteins (including type 1 collagen) results in the possibility of splicing causing diverse functional consequences. Hence, functional analysis plays a vital role in the interpretation of splicing variants and comprehension of their molecular impacts on protein structure and function. Splice variants are responsible for approximately 65% of the pathology in KD (4). The patient with the c.1023 + 2T > C splice variant had mild short stature, unlike the other KD patients. If a genotype-phenotype correlation for this variant is made, it may be associated with mild short stature, although this is speculative at present. More patient series are needed to make a clear assessment. The Gly855Asp variant was a novel variant. The substitution of glycine to aspartate has not been widely observed in the literature and is more commonly associated with ACG2/HCG and STL1. Severe short stature and pectus excavatum were remarkable in our patient.

The c.1394G > A (p.Gly465Asp) variant was associated with SEMD Strudwick type in the present study. This missense variant was also novel. In the literature, the substitution of glycine with alanine at position 465 has been classified as SEDC (15). The substitution of glycine with serine at the same position has also been demonstrated in the literature and was also associated with SEDC. It has been proposed that this replacement does not lead to severe short stature (8). The glycine to aspartate substitution reported here may result in more severe skeletal features, including severe short stature, pectus carinatum, marked lordosis, and atlanto-axial instability. In addition, cleft palate, hearing loss, and inguinal hernia were present as extra-skeletal findings. Another novel variant c.2006G > C (p.Gly669Ala) has been associated with SEDC. Markova et al. (16) reported a patient with a glycine to serine conversion at the same position. This patient had severe short stature (-9.7 SDS) and myopia and was diagnosed with SEDC. Our patients were also diagnosed with SEDC. They had severe short stature but did not have extra-skeletal manifestations, such as visual or hearing impairments. In studies involving patients diagnosed with

SEDC and glycine to alanine conversions, none of them exhibited extra-skeletal manifestations, contrast to the patients presented herein (17,18,19). Furthermore, glycine to alanine conversions has been described as extremely rare, and to the best of our knowledge, it is most commonly associated with SEDC. It may be that SEDC patients with a glycine to alanine conversion may not exhibit extra-skeletal manifestations. However, additional studies are needed to confirm this. Mild manifestations of SED are frequently (mis) diagnosed as "rheumatoid-like" arthritis or "degenerative conditions" affecting the hip joints (16). Therefore, early-onset joint pain and a family history of joint prostheses should raise suspicion for type 2 collagenopathies.

As is the case with other collagenopathies, patients with the *COL2A1* variant should be monitored with a multidisciplinary approach. Atlantoaxial dislocation, odontoid hypoplasia, and retinopathy may be life-threatening or result in significant morbidity. From the perspective of pediatric endocrinology, these patients may present with short stature of varying clinical severity, which may be classified as idiopathic short stature or skeletal dysplasia and monitored accordingly. From the perspective of pediatric endocrinology, as the patients will be monitored by endocrinologists until they reach adulthood, it is important to enhance awareness and understanding of potential comorbidities, multidisciplinary management strategies, and future treatment options. Patients with skeletal dysplasia tend to be referred to pediatric endocrinology for short stature and pediatric rheumatology for joint problems. The phenotypic features in this small patient group showed that the severity of short stature was variable and increased with age. Mild cases presenting at a young age, like patient 1, may present with mild short stature and be considered idiopathic short stature. However, the presence of dysmorphic findings, such as short neck, lordosis, and absence of pubic ossification, which were observed in all patients in our cohort, may assist in the targeted planning of molecular studies by suggesting the possibility of chondrodysplasia due to *COL2A1*.

These patients represent a group for whom there is limited experience with growth hormone (rhGH) therapy. Concerns have been raised about the potential for skeletal disproportion, scoliosis, and retinal complications, in addition to the limited benefit in terms of height gain with rhGH. A recent study from China published in 2022 investigated the efficacy of rhGH treatment in nine collagenopathy patients, two of whom had treatment combined with GnRHa. The results indicated an improved growth rate and height SDS; however, it was concluded that close monitoring of adverse reactions such as scoliosis is required. Among the five patients with a confirmed *COL2A1* mutation who received growth hormone therapy, there was a mean improvement of approximately +0.61 in height SDS over the course of one year (20). Future studies in collagenopathy patients, whose numbers are increasing with the increase in molecular diagnostic possibilities, will focus on the long-term morbidity and response to rhGH and alternative treatment options. Another potential concern is the possibility of early-onset osteoporosis. Type 2 collagen alpha 1, encoded by *COL2A1*, is the primary collagen found in articular cartilage and is synthesized by chondrocytes. *COL2A1* variants have been linked to an impairment of bone microstructure, which can lead to early onset osteoporosis, skeletal dysplasia, and osteoarthritis. In some cases, the dysplasia component may be so mild that only the pseudorheumatoid joint problems come to clinical attention. Therefore, a skeletal assessment is essential to accurately identify the condition and develop an optimal therapeutic strategy. Early diagnosis may prevent a progressive decline in bone mass, as antiresorptive treatment represents a valuable treatment option (21).

We report four novel variants in the *COL2A1* gene, expanding the molecular spectrum of type 2 collagenopathies. Analyzing the clinical and genetic aspects of different types of type 2 collagenopathies can enhance our comprehension of the underlying mechanisms and enable more precise prediction of their progression in the early stages. This, in turn, can lead to improved medical treatment and quality of life for affected individuals. In contrast to the past, the possibility of diagnosing rare genetic diseases has increased as genetic tests have become cheaper and more accessible. Furthermore, precise identification of type 2 collagenopathies is important to provide accurate genetic counseling. Patients should be offered pre-implantation genetic testing because of the notable risk of recurrence.

Study Limitations

This study was conducted with a limited number of cases. Furthermore, functional studies are required to confirm the pathogenicity of the identified variants.

Conclusion

Short stature is one of the most common conditions encountered in clinical practice. In skeletal dysplasias, *COL2A1*-related diseases are relatively common. We described six patients with type 2 collagenopathies, four of whom had novel variants, including three with KD, two with SEDC, and one with SEDC Strudwick type. Identification of novel variants is key to expanding the range of genetic variations in diseases and in gaining a clearer understanding of the relationship between genotype and phenotype in larger patient cohorts. The similarity of clinical and radiological findings and the variability of phenotype in

COL2A1-related diseases make it challenging to categorize patients.

Ethics

Ethics Committee Approval: The study adhered to the Declaration of Helsinki and was approved by the Ethical Committee of ***University of Health Sciences Türkiye, Ümraniye Training and Research Hospital*** (approval number: B.10.1.TKH.4.34.H.GP.0.01/293, date: 05.09.2024).

Informed Consent: Written informed consent for genetic testing and publication of clinical findings, patient photographs, and molecular results was obtained from each patient or their parents.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Burcu Yeter, Concept: Burcu Yeter, Yasemin Kendir Demirkol, Ahmet Hamdi Akgülle, Design: Burcu Yeter, Ahmet Hamdi Akgülle, Data Collection or Processing: Metin Eser, Yasemin Kendir Demirkol, Betül Sözeri, Analysis or Interpretation: Burcu Yeter, Yasemin Kendir Demirkol, Literature Search: Burcu Yeter, Yasemin Kendir Demirkol, Heves Kırmızıbekmez, Writing: Burcu Yeter, Yasemin Kendir Demirkol, Heves Kırmızıbekmez.

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