ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2024.2022-12-8

J Clin Res Pediatr Endocrinol 2024;16(4):431-442

Molecular Genetic Diagnosis with Targeted Next Generation Sequencing in a Cohort of Turkish Osteogenesis Imperfecta Patients and their Genotype-phenotype Correlation

Samim Özen[1], Damla Gökşen[1], Ferda Evin[2], Esra Işık[3], Hüseyin Onay[4], Bilçağ Akgün[4], Aysun Ata[1], Tahir Atik[3], Füsun Düzcan[5], Ferda Özkınay[3], Şükran Darcan[1], Özgür Çoğulu[3]

[1]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, İzmir, Turkey
[2]Bakırçay University, Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology, İzmir, Turkey
[3]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Genetics, İzmir, Turkey
[4]Ege University Faculty of Medicine, Department of Medical Genetics, İzmir, Turkey
[5]Tınaztepe University Faculty of Medicine, Department of Medical Genetics, İzmir, Turkey

What is already known on this topic?

Variants in *COL1A1* and *COL1A2* genes, encoding type 1 collagen, are responsible for most of the etiology in osteogenesis imperfecta (OI). Molecular diagnosis is useful for early diagnosis, estimating the prognosis, determination of other individuals in the family and choice of treatment according to knowledge of variable responses to drugs.

What this study adds?

By using a targeted OI next-generation sequencing (NGS) panel *(COL1A1, COL1A2, IFITM5, SERPINF1, CRTAP, P3H1, PPIB, SERPINH1, FKBP10, SP7, BMP1, MBTPS2, PLOD2)*, the detection rate of disease causing variants was as high as 82.1% *(COL1A1, COL1A2, P3H1, SERPINF1, FKBP10)* in pediatric patients. We believe that targeted NGS was a valuable method for genetic diagnosis in pediatric patients with OI.

Abstract

Objective: Osteogenesis imperfecta (OI) consists of a group of phenotypically and genetically heterogeneous connective tissue disorders that share similar skeletal anomalies causing bone fragility and deformation. The aim was to investigate the molecular genetic etiology and determine the relationship between genotype and phenotype in OI patients using targeted next-generation sequencing (NGS).

Methods: A targeted NGS analysis panel (Illumina TruSight One) containing genes involved in collagen/bone synthesis was performed on the Illumina Nextseq550 platform in patients with a confirmed diagnosis of OI.

Results: Fifty-six patients (female/male: 25/31) from 46 different families were included. Consanguinity was noted in 15 (32.6%) families. Based on Sillence classification 18 (33.1%) were type 1 OI, 1 (1.7%) type 2, 26 (46.4%) type 3 and 11 (19.6%) type 4. Median body weight was -1.1 (-6.8, - 2.5) standard deviation scores (SDS), and height was -2.3 (-7.6, - 1.2) SDS. Bone deformity affected 30 (53.5%), while 31 (55.4%) were evaluated as mobile. Thirty-six (60.7%) had blue sclera, 13 (23.2%) had scoliosis, 12 (21.4%) had dentinogenesis imperfecta (DI), and 2 (3.6%) had hearing loss. Disease-causing variants in *COL1A1* and *COL1A2* were found in 24 (52.1%) and 6 (13%) families, respectively. In 8 (17.3%) of the remaining 16 (34.7%) families, the NGS panel revealed disease-causing

Cite this article as: Özen S, Gökşen D, Evin F, Işık E, Onay H, Akgün B, Ata A, Atik T, Düzcan F, Özkınay F, Darcan Ş, Çoğulu Ö. Molecular Genetic Diagnosis with Targeted Next Generation Sequencing in a Cohort of Turkish Osteogenesis Imperfecta Patients and their Genotype-phenotype Correlation. J Clin Res Pediatr Endocrinol. 2024;16(4):431-442

Address for Correspondence: Ferda Evin MD, Bakırçay University, Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology and Diabetes, İzmir, Turkey
E-mail: ferdaevin88@gmail.com **ORCID:** orcid.org/0000-0001-7169-890X

Received: 19.01.2023
Accepted: 29.05.2024
Epub: 03.06.2024
Publication date: 04.12.2024

J Clin Res Pediatr Endocrinol
2024;16(4):431-442

variants in three different genes (*FKBP10, SERPINF1*, and *P3H1*). Nine (23.6%) of the variants detected by NGS panel had not previously been reported and were also classified as pathogenic based on American College of Medical Genetics guidelines pathogenity scores. In ten (21.7%) families, a disease-related variant was not found in any of the 13 OI genes on the panel.
Conclusion: Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis. Furthermore, nine new variants were identified in known OI genes which were classified as pathogenic by standard guidelines.
Keywords: Osteogenesis imperfecta, next-generation sequencing, *COL1A1*, genetics

Introduction

Osteogenesis imperfecta (OI) is a hereditary disease of connective tissue characterized by increased bone fragility and multiple fractures (1,2,3). OI is a rare disorder with a frequency of 1/15,000-20,000. This generalized connective tissue disorder has an important effect on bone structure, leading to skeletal fragility and developmental delay. Clinical severity varies between the types of OI and additional features such as dentinogenesis imperfecta (DI), blue sclerae, short stature, hearing loss, and cardiac malformations may be present (4). Variants in the collagen genes are responsible for approximately 85-90% of OI. Patients with OI have heterogeneous clinical and genetic features (5). Thus, clinical diagnosis is insufficient for optimal diagnosis, management, prognosis, and genetic counseling. In recent years, new OI types have been discovered with the development of improved genetic analysis techniques. The disorder can also be caused by variants of genes related to collagen structure and function (6,7). The most recently identified genes today are characterized by primary defects in osteoblast differentiation (2,3,6,8).

Advances in next-generation sequencing (NGS) technology have enabled the discovery of novel genes and pathogenic variants related to OI (2,9,10). Molecular diagnosis is useful for early diagnosis, prognosis, identifying other individuals in the family with the same variants, and deciding on optimal treatment based on the published evidence (2,9,10,11).

In this study, the aim was to investigate the molecular genetic etiology of OI using a targeted NGS panel and to determine the genotype-phenotype relationship in OI patients, and the effectiveness of this genetic panel for diagnosis.

Methods

Study Group

A cohort of clinically and/or radiologically diagnosed OI patients followed in Ege University Faculty of Medicine Pediatric Endocrinology and Diabetes Department were included in the study. Inclusion criteria were patients between 0-18 years of age with unknown molecular genetic etiology. Patients having any genetic disease other than OI that could cause bone fragility and other chronic diseases or patients with fragile bone syndrome due to medication, such as steroids or chemotherapy, were excluded.

Demographic data (age, gender, consanguinity, family history), clinical features (OI subgroup, frequency of annual bone fractures, treatment procedure and response), physical examination findings (bone deformities), and bone radiography findings were obtained from hospital records. Patients' weight and height and their standard deviation (SD) scores (SDS) were calculated based on Turkish standards (12,13).

The study was approved by the Ethics Committee of Ege University Faculty of Medicine (ethic committee number: 18-3.1/55, date: 20.03.2018), and samples from the patients were obtained in accordance with the Helsinki Declarations. Written informed consent for molecular analysis was obtained from all cases or their parents/guardians.

Molecular Analysis

Genomic DNA samples were extracted from leukocytes from 1 mL of peripheral blood obtained from all patients using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. DNA quality and quantity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For sequence analysis, a targeted NGS panel (TruSight One Panel by Illumina®) including 13 genes (*COL1A1, COL1A2, IFITM5, SERPINF1, CRTAP, P3H1, PPIB, SERPINH1, FKBP10, SP7, BMP1, MBTPS2, PLOD2)* associated with OI was used.

Data Analysis

Sequencing data was analyzed using Illumina VariantStudio software and IGV (Integrative Genomics Viewer). Firstly, 13 genes known to be responsible for OI were analyzed. Variants in these genes with a frequency of less than 0.5% in public databases including NCBI dbSNP build155 (http://www.ncbi.nlm.nih. gov/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), gnomAD (https://gnomad.broadinstitute.org/) and NHLBI Exome Sequencing Project Exome Variant Server (http://evs.gs.washington.edu/EVS/) were selected. The impact of the variants on the protein

structure was identified using several *in silico* prediction tools, such as MutationTaster, PolyPhen-2, and SIFT. The conservation of residues across species was evaluated by the PhyloP algorithm and GERP (14,15,16,17). The pathogenicity of all variants identified was classified according to the American College of Medical Genetics (ACMG) recommendations. The pathogenity scores were obtained from the https://www.acmg.net/ website. The ACMG Guidelines were established by clinicians and clinical lab directors who are experts in clinical genetics and members of the ACMG, the Association for Molecular Pathology (AMP), and/or the College of American Pathologists. Franklin Genoox software and database was used for ACMG Classification. There are 28 criteria in the ACMG guidelines. During variant interpretation, variants are classified into five tiers: pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign, and benign (B), depending on the applicable criteria. These criteria can be classified by the weight and type of evidence indicated by each criterion. The 28 criteria can be classified into eight types: population data, computational data, functional data, segregation data, *de novo* data, allelic data, other databases, and other data, depending on the source of evidence (18).

Confirmation

The most likely disease-causing variants, identified by data analysis, were confirmed using direct Sanger sequencing on ABI PRISM 3130 DNA analyzer (Applied Biosystems) and Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit (Life Technologies) (Fisher Scientific - Göteborg - Sweden).

Statistical Analysis

Analysis was conducted using Statistical Package for the Social Sciences for Windows, version 25.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics are reported as mean ± SD for normally distributed variables and median (range) for skewed data. Groups were compared using independent samples t-test for normally distributed variables and the Mann-Whitney U test for skewed data. Trends across time were analyzed using linear polynomial contrasts (ANOVA). A $p < 0.05$ was considered statistically significant. No adjustment was made for a multiplicity of statistical tests.

Results

Clinical Manifestations

Fifty-six patients (female/male: 25/31) from 46 different families were included in the study. In 15 (32.6%) families, consanguineous marriage was noted. The mean age of the cohort on admission was 4.5 ± 3.7 years, median body weight was -1.1 (-6.8, - 2.5) SDS, and height was -2.3 (-7.6, - 1.2) SDS. Based on the actualized Sillence classification (Table 1), 18 (33.1%) patients were considered to be type 1, 1 (1.7%) type 2, 26 (46.4%) type 3, and 11 (19.6%) type 4 (19). Bone deformity was detected in 30 (53.5%) of the patients, while 31 (55.4%) were evaluated as mobile. Thirty-six (60.7%) patients had blue sclera, 13 (23.2%) had scoliosis, 12 (21.4%) had DI, and 2 (3.6%) had hearing loss.

Molecular Analysis Findings

Sequence analysis of the *COL1A1* and *COL1A2* genes revealed heterozygous variants in 24 (52.1%) and 6 (13%) families, respectively. The (NM_000088.4(*COL1A1*):c.3677A > G/p.(Asp1226Gly)/rs1319157667) variant was detected together with the c.2296G > C variant in *COL1A2* in one patient. The variant detected in the *COL1A1* gene was also found in the asymptomatic father of the patient in segregation analysis. Based on this family history, she was excluded from the *COL1A1* group. The remaining 16 families were molecularly analyzed using the NGS panel, and in 8 (17.3%) families, a disease-causing variant in three different genes (*FKBP10, P3H1*, and *SERPINF*) was identified. Nine (23.6%) of the detected variants in all genes have not been previously reported and were considered to be deleterious based on prediction tools. Following a two-step NGS-based molecular analysis, a molecular diagnosis was achieved in 38 (82.6%) families in the study group.

Genotype-phenotype Relations

Fifteen of the *COL1A1* variants were boys, and 14 were girls. The mean age at admission was 4.69 ± 3.66 years, and weight and height SDS were -0.73 ± 1.39 and - 2.41 ± 4.45, respectively. The distribution of clinical diagnosis was as follows: 13 (44.8%) type 1; 1 (3.4%) type 2; 10 (34.4%) type 3; and 5 (17.2%) type 4. Recurrent pathological fractures

Table 1. The actualized Sillence classification (20)

OI type	
1	Mild form. Patients have no bone deformities, normal or near normal stature.
2	Extremely severe form is perinatal lethal.
3	Most severe form in children surviving the neonatal time, severely deforming, extreme short stature.
4	Intermediate form between type 1 and type 3: mild to moderate bone deformities and variable short stature.

OI: osteogenesis imperfecta

were detected in 25 (86.2%) of the patients, and deformity of extremities in 7 (24.1%) patients. Six (20.6%) patients were mobile with help or had the ability to sit. The rest of the patients were completely mobile. Twenty-five patients (86.2%) had blue sclera, and 8 (27.5%) had DI.

The mean age of admission of those with variants in the *COL1A2* gene (n = 6, 4 girls) was 5.17 ± 3.45 years, weight SDS was -2.86 ± 2.33, and height SDS was -3.07 ± 1.01. The distribution of clinical types was four (66.6%) type 3 and 2 (33.3%) type 4. In all patients, 2 or more recurrent pathological bone fractures and deformities were detected. In 5 (50%) patients, blue sclera and DI was found in one (16.6%) patient.

Biallelic variants in the *SERPINF1* gene were detected in four patients (patients 36, 37, 38, and 39) from three families. In one case, compound heterozygous variant c.80dupA/c.907C > T was present, and this patient's OI phenotype was compatible with type 3 with severe deformities, recurrent fractures, and short stature. However, family segregation was not performed in this patient.

A homozygous novel c.446T > G p.(Leu149Arg) variant was detected in the *P3H1* gene in a 0.2-year-old patient with a history of consanguineous marriage. The patient's weight SDS was -0.36, and height SDS was -2.12.

A homozygous c. 15dupC variant was identified in the *FKBP10* gene in a male patient with Bruck syndrome and clinically type 4 OI. Her parents were heterozygous for the same variant. The same variant was demonstrated in the sibling of the index case with a similar phenotype. These cases had severe OI and congenital contractures of large joints, short stature, and scoliosis.

Genotype and phenotype characteristics of patients with OI-related variants are given in Tables 2 and 3.

Discussion

The present study investigated the molecular etiology of 56 clinically diagnosed OI patients from 46 different families using an NGS panel, including a total of 4800 genes, including 13 genes related to OI. Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis with this TruSight One Panel. Such targeted gene panels are extremely reliable and validated and can be used in a wide range of indications for genetic diseases. Panels containing the genes of most diseases inherited as Mendelian in humans, such as the Illumina TruSight One Panel, are also referred to as "clinical exomes". This expression should not be confused with whole exome sequencing (WES) because approximately 20,000 genes detected in mankind are analyzed by WES analysis, while the clinical exome only contains genes associated with disease in humans. The advantage of the TruSight One panel compared to WES is that it is easier to analyze the results, and the cost is lower (20).

It is generally accepted that in-frame, partial deletions in the *COL1A1* or *COL1A2* genes can result in a lethal or severe OI phenotype when the resulting abnormal protein is not rapidly degraded but instead is incorporated in the triple helix exerting a dominant negative effect. In a study by (21), multiplex ligation-dependent probe amplification analysis was performed in the analysis of the *COL1A1* gene in a group of 106 index patients. These authors found seven patients with deletion of the complete *COL1A1* gene on one allele. In the present study, we did not evaluate the gross deletions and duplications in exons because this was outside the scope of the analysis methods. Although these types of variants are generally identified at low rates, they may be more relevant for the subgroup without any variants, in terms of disease severity through haploinsufficiency of *COL1A1* and *COL1A2* genes.

Consanguineous marriage was present in 28.5% of patients, and 39.3% had a family history of OI. Consanguineous marriage may lead to a high rate of autosomal recessive variants being found. In a study in India with seven patients of consanguineous marriage, *SERPINF1, PPIB*, and *CRTAB* mutations were detected (22). In a study evaluating *COL1A1* and *COL1A2* gene variants in 364 patients of Italian origin, the rate of positive family history rate was 57.7% (23). However, this increased rate of more than half may have been present because, with the exception of the two autosomal dominant gene variants in *COL1A1* and *COL1A2*, other types of OI were not studied. Family history rates were reported to be 53% in the Korean population (24), and 32-33% in different societies (25,26,27). The reason for these differences may be due to genetic differences in societies, variations in the genetic analysis methods used, and the changing frequency of *de novo* variants. Furthermore, the frequency of consanguineous marriage and/or founder variants in a society will lead to variability in the distribution of genes responsible for OI.

The clinical finding of blue sclera is one of the distinctive clinical features of OI and is frequently observed in patients with type 1 OI. Patients with type 3 and 4 OI may have blue sclera at birth, but the bluish color disappears with increasing age (2,8,28). In our study group, 34 (60.7%) of the patients had blue sclera.

Table 2. Genotype and phenotype characteristics of patients with variants related to osteogenesis imperfecta

Gene	Variant c.DNA (protein)	Consanguineous marriage in parents	Clinical type	Currently mobilization status	Number of fractures/ years	Bone deformity	BS	HL	DI	Patient number/ gender	Diagnosis age (yrs)
COL1A1	c.120C > A (p.Cys40*)	No	Type 3	Mobile	3	Yes, lower extremity	+	-	-	1/F	8.3
COL1A1	c.1283delG (p.Gly428Valfs*113)	No	Type 1	Mobile	3	No	+	-	-	2/F	1.8
			Type 1	Mobile	No	No	+	-	-	3/F	1.2
			Type 1	Mobile	3	No	+	-	-	4/M	6.7
COL1A1	c.1699C > T (p.Pro567Ser)	No	Type 4	Mobile	3	Yes, lower extremity	+	-	+	5/M	2.4
			Type 4	Mobile	No	No	+	-	+	6/M	6.1
COL1A1 / *COL1A2*	c.3677A > G (p.Asp1226Gly)/ c.2296G > C (p.Gly766Arg)	Yes	Type 4	Mobile	2	Yes, lower extremity	+	-	-	7/F	2.5 months
COL1A1	c.626G > A (p.Gly209Asp)	Unknown	Type 3	Assisted walking	3	Yes, very severe	+	-	-	8/M	8.5 months
COL1A1	c.1057-2A > C	Yes	Type 3	Mobile	6	No	+	-	-	9/M	3.7
COL1A1	c.1081C > T (p.Arg361*)	No	Type 1	Mobile	3	No	+	-	-	10/F	3.6
COL1A1	c.1299 + 1G > A	No	Type 1	Mobile	3	No	+	-	-	11/M	12.8
COL1A1	c.1299 + 1G > T	No	Type 1	Mobile	4	Yes, lower extremity	+	-	-	12/M	7.9
COL1A1	c.1353 + 2T > C	No	Type 3	Assisted walking	2	Yes, lower extremity	+	-	-	13/F	1.5 months
COL1A1	c.1405C > T (p.Arg469*)	No	Type 1	Mobile	2	No	+	-	+	14/F	3.4
COL1A1	c.2596G > A (p.Gly866Ser)	No	Type 2	Sitting	1	No	+	-	-	15/F	1.5 months
COL1A1	c.3235G > A (p.Gly1079Ser)	No	Type 3	Mobile	3	No	+	-	-	16/M	7.8
			Type 3	Mobile	3	No	+	-	-	17/M	10 months
COL1A1	c.3505G > A (p.Gly1169Ser)	No	Type 3	Assisted walking	3	Yes, lower extremity	+	-	-	18/F	10.6
COL1A1	c.1128delT (p.Gly377Alafs*164)	No	Type 1	Mobile	2	No	+	-	-	19/F	3.0
			Type 1	Mobile	3	No	+	-	-	20/M	2.9
COL1A1	c.1459_1460insA (p.Arg487Glnfs*6)	No	Type 1	Mobile	2	No	+	-	+	21/F	8.5 months
COL1A1	c.958G > C (p.Gly320Arg)	No	Type 4	Mobile	3	No	-	-	-	22/M	9.5
COL1A1	c.4051C > T (p.Gln1351*)	No	Type 3	Mobile	4	No	-	-	-	23/M	10.2
COL1A1	c.441delC (p.Gly148Aspfs*117)	No	Type 1	Mobile	3	No	+	-	+	24/M	7.1
COL1A1	c.886G > T (p.Gly296*)	No	Type 4	Mobile	2	No	+	-	-	25/F	3.9
COL1A1	c.1156-1G > A	Yes	Type 1	Mobile	1	No	+	-	-	26/F	4.6
COL1A1	c.3647A > G (p.Tyr1216Cys)	No	Type 4	Mobile	2	No	-	-	-	27/F	2.7
COL1A1	c.608G > T (p.Gly203Val)	No	Type 3	Sitting	5	Yes, lower extremity	+	-	+	28/M	1.7
COL1A1	c.1405C > T (p.Arg469*)	No	Type 1	Mobile	5	No	+	-	+	29/M	9.7
COL1A1	c.2829 + 2dupT	No	Type 3	Mobile	2	No	+	-	-	30/F	1.1
COL1A2	c.1972G > A (p.Gly658Ser)	No	Type 3	Assisted walking	4	Yes, lower extremity	-	-	-	31/F	10.3
COL1A2	c.3250G > T (p.Gly1084Cys)	No	Type 4	Assisted walking	2	Yes, lower extremity	+	-	-	32/M	10 months

Table 2. Continued

Gene	Variant c.DNA (protein)	Consanguineous marriage in parents	Clinical type	Currently mobilization status	Number of fractures/ years	Bone deformity	BS	HL	DI	Patient number/ gender	Diagnosis age (yrs)
COL1A2	c.928G > C (p.Gly310Arg)	Yes	Type 3	Assisted sitting	3	Yes, lower and upper extremity	+	-	+	33/M	3.5 months
COL1A2	c.1081G > A (p.Gly361Ser)	No	Type 3	Assisted walking	3	Yes, spine	-	-	-	34/F	5.7
COL1A2	c.3014G > A (p.Arg1005His)	No	Type 3	Immobile	3	Yes, very severe	-	+	-	35/F	6.5
SERPINF1	c.80dupA (p.Glu28Glyfs*37)/ c.907C > T (p.Arg303*)	No	Type 3	Immobile	4	Yes, very severe	-	-	-	36/F	8.5 months
SERPINF1	c.317G > C (p.Arg106Pro)	Yes	Type 1	Mobile	2	No	+	-	-	37/F	8.6
SERPINF1	c.988C > T (p.Gln330*)	Yes	Type 1	Mobile	2	No	+	-	-	38/M	11.6
			Type 3	Assisted sitting	4	Yes, spine	-	-	-	39/F	6 months
P3H1	c.446T > G (p.Leu149Arg)	Yes	Type 4	Mobile	4	Yes, lower extremity	-	-	-	40/F	2.5 months
P3H1	c.446T > G (p.Leu149Arg)	Yes	Type 3	Assisted walking	3	Yes, lower and upper extremity	-	-	-	41/F	1.5 months
FKBP10	c.1490G > A (p.Trp497*)	No	Type 4	Assisted walking	4	Yes, lower and upper extremity	-	-	-	42/M	4.3
			Type 4	Sitting	3	Yes, lower and upper extremity	-	-	-	43/M	5 months
FKBP10	c.831dupC (p.Gly278Argfs*95)	Yes	Type 3	Assisted sitting	3	Yes, lower and upper extremity	-	-	+	44/M	4.6
FKBP10	c.21dupC (p.Ser8Glnfs*67)	No	Type 3	Immobile	3	Yes, very severe	-	-	-	45/F	8.3
			Type 3	Assisted sitting	5	Yes, very severe	-	-	-	46/M	4.7
---	---	Yes	Sitting	Sitting	4	Yes, lower and upper extremity	+	-	-	47/F	6.2
---	---	Yes	Assisted sitting	Assisted sitting	3	Yes, very severe	-	-	-	51/M	3.7
---	---	Yes	Assisted sitting	Assisted sitting	3	Yes, very severe	+	-	-	53/M	1.5
---	---	No	Sitting	Sitting	3	Yes, lower extremity	-	-	-	48/M	13.2
---	---	No	Mobile	Mobile	1	No	+	-	-	49/M	10.6
---	---	No	Mobile	Mobile	3	Yes, upper extremity and spine	-	-	-	50/M	2.2
---	---	Yes	No sitting	No sitting	2	Yes, lower extremity	-	+	-	52/M	2.1
---	---	No	Mobile	Mobile	2	No	-	-	-	54/M	2.4
---	---	Yes	Mobile	Mobile	4	No	+	-	+	55/M	5.2
---	---	Yes	Assisted sitting	Assisted sitting	1	Yes, very severe	-	-	+	56/F	1.5 months

BD: bone deformity, BS: blue sclera, DI: dentinogenesis imperfecta, HL: hearing loss, M: male, F: female, yrs: years

Table 3. Genetic characteristics of patients with variants related to osteogenesis imperfecta

Gene	Variant c.DNA (protein)	Transcript	Genomic position	dbSNP	ACMG/AMP criteria	ExAC	GnomAD (aggregated)
COL1A1	c.120C > A (p.Cys40*)	NM_000088.4	chr17-48277292	rs762780039	P	N/A	N/A
COL1A1	c.1283delG (p.Gly428Valfs*113)	NM_000088.4	chr17-48272608		LP	N/A	N/A
COL1A1	c.1699C > T (p.Pro567Ser)	NM_000088.4	chr17-48271372		VUS	N/A	N/A
COL1A1	c.3677A > G (p.Asp1226Gly)/	NM_000088.4	chr17-48264138	rs1319157667	VUS	N/A	0.0032
COL1A2	c.2296G > C (p.Gly766Arg)	NM_000089.4	chr7-94050321		P		
COL1A1	c.626G > A (p.Gly209Asp)	NM_000088.4	chr17-48275326		P	N/A	N/A
COL1A1	c.1057-2A > C	NM_000088.4	chr17-48273028	rs66511271	LP	N/A	N/A
COL1A1	c.1081C > T (p.Arg361*)	NM_000088.4	chr17-48273002	rs72645366	P	N/A	N/A
COL1A1	c.1299 + 1G > A	NM_000088.4	chr17-48272592	rs66490707	P	N/A	N/A
COL1A1	c.1299 + 1G > T	NM_000088.4	chr17-48272592		LP	N/A	N/A
COL1A1	c.1353 + 2T > C	NM_000088.4	chr17-48272406	rs72648335	LP	N/A	N/A
COL1A1	c.1405C > T (p.Arg469*)	NM_000088.4	chr17-48272138	rs762428889	P	N/A	N/A
COL1A1	c.2596G > A (p.Gly866Ser)	NM_000088.4	chr17-48267237	rs67445413	P	N/A	N/A
COL1A1	c.3235G > A (p.Gly1079Ser)	NM_000088.4	chr17-48265483	rs72654802	P	N/A	N/A
COL1A1	c.3505G > A (p.Gly1169Ser)	NM_000088.4	chr17-48264402	rs67815019	P	N/A	N/A
COL1A1	c.1128delT (p.Gly377Alafs*164)	NM_000088.4	chr17-48272954	rs72645370	P	N/A	N/A
COL1A1	c.1459_1460insA (p.Arg487Glnfs*6)	NM_000088.4	chr17-48272083		LP	N/A	N/A
COL1A1	c.958G > C (p.Gly320Arg)	NM_000088.4	chr17-48273560		LP	N/A	N/A
COL1A1	c.4051C > T (p.Gln1351*)	NM_000088.4	chr17-48263336		P	N/A	N/A
COL1A1	c.441delC (p.Gly148Aspfs*117)	NM_000088.4	chr17-48276616	rs1473458290	P	N/A	N/A
COL1A1	c.886G > T (p.Gly296*)	NM_000088.4	chr17-48273862		LP	N/A	0
COL1A1	c.1156-1G > A	NM_000088.4	chr17-48272840		LP	N/A	N/A
COL1A1	c.3647A > G (p.Tyr1216Cys)	NM_000088.4	chr17-48264168	rs1555571849	LP	N/A	N/A
COL1A1	c.608G > T (p.Gly203Val)	NM_000088.4	chr17-48275344	rs72667031	P	N/A	N/A
COL1A1	c.1405C > T (p.Arg469*)	NM_000088.4	chr17-48272138	rs762428889	P	N/A	N/A
COL1A1	c.2829 + 2dupT	NM_000088.4	chr17-48266735		LP	N/A	N/A
COL1A2	c.1972G > A (p.Gly658Ser)	NM_000089.4	chr7-94047811		LP	N/A	N/A
COL1A2	c.3250G > T (p.Gly1084Cys)	NM_000089.4	chr7-94056590		P	N/A	N/A
COL1A2	c.928G > C (p.Gly310Arg)	NM_000089.4	chr7-94038912	rs72656391	LP	N/A	N/A

Table 3. Continued

Gene	Variant c.DNA (protein)	Transcript	Genomic position	dbSNP	ACMG/AMP criteria	ExAC	GnomAD (aggregated)
COL1A2	c.1081G > A (p.Gly361Ser)	NM_000089.4	chr7-94039599		LP	N/A	N/A
COL1A2	c.3014G > A (p.Arg1005His)	NM_000089.4	chr7-94055751	rs200357942	VUS	N/A	N/A
SERPINF1	c.80dupA (p.Glu28Glyfs*37)/ c.907C > T (p.Arg303*)	NM_002615.7	chr17-1670283		LP	0.0025	0.0046
			chr17-1679946	rs763291398	P		
SERPINF1	c.317G > C (p.Arg106Pro)	NM_002615.7	chr17-1674356	rs148872301	VUS	N/A	N/A
SERPINF1	c.988C > T (p.Gln330*)	NM_002615.7	chr17-1680027		LP	0.0016	0.0008
P3H1	c.446T > G (p.Leu149Arg)	NM_022356.4	chr1-43232197		VUS	0.0016	0.0008
FKBP10	c.1490G > A (p.Trp497*)	NM_021939.4	chr17-39977996		LP	N/A	N/A
FKBP10	c.831dupC (p.Gly278Argfs*95)	NM_021939.4	chr17-39975558	rs137853883	P	N/A	N/A
FKBP10	c.21dupC (p.Ser8Glnfs*67)	NM_021939.4	chr17-39969300	rs782271121	P	0.0189	0.0107

*Exome Aggregation Consortium (http://exac.broadinstitude.org).
#The allele frequency in the ExAC database does not represent all ethnic group.
LP: likely pathogenic, VUS: variant of unknown significance, P: pathogenic, N/A: not applicable, ACMG: American College of Medical Genetics, AMP: Association for Molecular Pathology

Bone fractures and deformities in OI usually occur at an early age and are often caused by repeated bone remodeling in long bones (2,29). This affects patients' growth, functional status, and mobility. In our study, 20.6% of patients with the *COL1A1* variant and all of those with the *COL1A2* variant had difficulty walking. In 24.1% of patients with the *COL1A1* variants, and all of those with the *COL1A2* variants, deformities were detected in the extremities. Mohd Nawawi et al., (30) showed that 63.6% of all OI patients had bone deformities at the age of nine years and needed help to walk. Studies have shown that bone deformities are more common in patients with qualitative variants than quantitative variants (2,29). Hald et al. (31,32) showed that OI patients with quantitative defects had normal protein structure in bone, despite collagen deficiency. This allows bone mineralization and thus leads to fewer breakages than qualitative defects (11,31).

DI has been reported in more frequently type 3 and rarely type 1 OI (33,34). DI was detected in 12 (21.4%) of the patients in the present study. The clinical diagnosis of the patients with DI was as follows: 5 patients (41.6%) were type 1, 4 (33.3%) type 3, and 3 (25%) type 4. In another study, DI was reported to be more frequent in patients with more severe clinical types (type 3 and 4) than in moderately affected groups (type 1) (34). Those with a qualitative variant, that is a problem in collagen structure, are more at risk of developing DI. Structurally abnormal collagen affects the development of dental germ cells in the predentin during the mineralization process (35).

In the present study, 63.1% had a variant in the *COL1A1* gene, 13.1% in the *COL1A2* gene, and 2.6% in both genes; in total, 78.8% of patients had variants in these two genes. In 11 (19.6%) out of 21 patients without variants in these genes, by NGS analysis, three other gene (*SERPINF1, FKBP10*, and *P3H1)* variants were detected. Three (7.8%) families had *FKBP10*, 3 (7.8%) families had *SERPINF1,* and 2 (5.2%) families had the *P3H1* variant. Abali et al. (36) studied 89 patients with OI. Similarly to our study, these authors reported a majority (61.4%) having variants in *COL1A1* and *COL1A2* genes, while much lower proportions had varinats in other OI-associated genes: 5 (5.6%) with *FKBP10*; 2 (2.2%) *LRP5*; 1 (1.1%) *P3H1*; 1 (1.1%) *CRTAP*; 1 (1.1%) *BMP1*; and 1 (1.1%) *SPARC* variant.

COL1A1 and *COL1A2* Gene Variants

Variants in *COL1A1* and *COL1A2* encoding type 1 collagen are responsible for most of the etiology in OI. In the present study, 62.4% of patients had variants in these two genes, similarly to previous repots where variants in these two genes were responsible for 51-73% of the disease (25,30,37,38).

Moreover, variants in the COL1A1 gene were detected more frequently in both the present study and earlier studies than the *COL1A2* gene (4,25,27,30).

In one (3.3%) case (patient 7), a heterozygous variant was detected in both *COL1A1* c.3677A>G p.(Asp1226Gly) and *COL1A2* c.2296G>C p.(Gly77Arg) genes. The variant detected in the *COL1A1* gene was also found in the asymptomatic father of the patient in segregation analysis and we made the decision to discount the VUS variant in the *COL1A1* gene as causing the phenotype. The variant in the *COL1A2* gene was previously reported as pathogenic. Therefore, this variant may be responsible for the clinical findings in this case, who had severe clinical type with recurrent fractures and severe deformities. No cases carrying variants in both these two genes simultaneously have been reported previously. However, Ji et al. (39) reported a case with a severe clinic variant in *COL1A1* and *SERPINF1*. Oligogenic inheritance should also be considered in cases with severe clinical features.

In patient 5, the variant c.1699C>T p.(Pro567Ser) in *COL1A1* gene. The variant c.1699C>T in COL1A1 was detected once in the GME Variome database and once in the Turkish Variome database. The frequency of the variant in GMA Variome was 0.05% and it was 0,02% in the Turkish Variome. Both frequencies are less than 0.06%, which is the cut-off level for ACMG-PM2 criteria for this gene, and this is supporting evidence for the possibly pathogenic nature of the variant. This variant has not been reported in association with OI or any disease.

P3H1 Gene Variants

A homozygous c.446T> G p.(Leu149Arg) variant was detected in the *P3H1* gene in two unrelated female patients (Patients 40 and 41) who were admitted with recurrent fractures before two months of age. This variant was thought to be disease-causing in *in silico* analysis and was not previously reported. Recurrent fractures continued with severe clinical phenotypes. In the literature, clinical OI type VIII due to *P3H1* gene variants have moderate/severe phenotypic features (31). In the West African community, in the *P3H1* gene, a relatively high c.1080+1G>T carriage was detected: 1/240. The homozygous form was associated with the perinatally lethal form of OI. This variant was thought to be a founding variant (40,41,42) but was not observed in any of the 56 patients in the present study.

SERPINF1 Gene Variants

In some populations, *SERPINF1* and *CRTAP* variants have been reported to be responsible for recessive OI types. Some variants have been suggested as causing a "founder" effect (43).

In the present study, four different variants were detected in three patients from different families, and another variant was detected in two siblings. Patients with *SERPINF1* variants had a more severe clinical picture and early admission. Compound heterozygous c.80dupA p.(Glu28Glyfs*37) / c.907C>T p.(Arg303Ter) variant was found in an infant (patient 36) with fractures from birth, widespread deformities, and severe short stature. In *in silico* analyses, both variants were disease-causing. No blue sclera, DI, or hearing loss was detected. In the follow-up, despite treatment, his fractures recurred, his deformities increased, and independent mobilization never developed. Another patient (patient 39) who presented with a severe clinical picture at the age of 6 months had a homozygous c.988C>T p.(Gln330Ter) variant. Vertebral and lower extremity fractures were present. Similar to these patients, most *SERPINF1* gene variants previously reported have been due to frameshift and nonsense variants (44,45). A missense homozygous c.317G>C p.(Arg106Pro) variant was found in a patient with a milder clinical picture who presented at 8.6 years with recurrent fractures without deformities. This variant was predicted to be a VUS by Franklin Genoox and Varsome programmes, by the ACMG 2015 criteria. Most of the predictions tool predicted that this variant will be pathogenic or VUS. This variant has low population frequency. Given this evidence, it was thought that this homozygous c.317G>C p.(Arg106Pro) variant detected in *SERPINF1* might be responsible for the clinical picture in the patient. These variants impair production of circulating pigment epithelium-derived factor (PEDF) as well as loss of PEDF protein function (2). Rauch et al. (46) reported that measuring PEDF concentration in serum may be a potential marker in assessing patients' clinical severity.

FKBP10 Gene Variants

A homozygous c.21dupC p.(Ser8Glnfs * 67) variant in the *FKBP10* gene was detected in an 8.3-year-old girl (patient 45) who presented with congenital joint contractures, recurrent fractures, and chest deformity resembling Bruck syndrome. No consanguineous marriage was reported. *FKBP10* gene variants have been associated with severe OI and Bruck syndrome (2,11). In two brothers with Bruck syndrome, Shaheen et al. (47) reported a homozygous 8-bp insertion variant in the *FKBP10* gene. Alanay and Krakow (48) reported that the patients in Shaheen et al.'s (47) study may have Bruck syndrome and that the clinical picture may be milder because they received bisphosphonate

treatment. Researchers have reported that different variants in the *FKBP10* gene can explain the variable severity of phenotypes.

Genotype-phenotype correlations in OI have been extensively studied over the years, with certain investigations revealing significant associations (49,50). Notably, more severe phenotypes have been observed in patients harboring pathogenic variants in *COL1A1* compared to those in *COL1A2* (51). Mrosk et al. (52) suggested a robust correlation between genotype and the severity of phenotypes. They proposed a ranking based on phenotype severity as follows: *P3H1, COL1A1*, and *COL1A2*, respectively. In the present study, 60% of the patients with variants detected in the *COL1A1* and *COL1A2* genes, 50% of the patients with variants detected in the *SERPINF1* and *P3H1* genes, and 60% of the patients with variants detected in the *FKBP10* gene had a severe phenotype. In addition, we identified a variant in a total of 15 affected individuals across seven families. Remarkably, the clinical types and features of cases with the same variant within these families were similar. On the other hand, c.1299+1G>A variant was detected in the *COL1A1* gene in cases 11 and 12 from different families, and c.446T>G variant was detected in the *P3H1* gene in cases 40 and 41. The clinical features of pateints 11 and 12 were similar. However, while case 41 was type 4 OI, case 41 was type 3 OI and case 41 showed more severe type features. The literature indicates that while varying phenotypes can exist within the same family, similarities can also exist among individuals from different families who share the same genetic variant (2,53). The genotype-phenotype relationship in OI remains variable, as carriers of the same variant may develop diverse phenotypes. Furthermore, the factors influencing additional phenotypic differences have yet to be fully elucidated.

In the present study, no variants were detected in any of the genes covered by TruSight One used in the targeted NGS analysis in 10 (17.8%) patients. There may be more OI-associated genes or perhpas new candidate genes that were not covered by this panel. Targeted gene panels are highly efficient in the diagnosis of genetic disorders, which have genetic heterogeneity. This panel gave an 82% diagnostic yield. For most of the cases this high diagnostic yield was clinically useful but current technology allows us to perform WES-CNV at a comparable price. However, in most centers the capacity of the genetic laboratories is the main determinant of the genetic approach.

In one study, it was noted that an unusually high percentage of AR forms due to mutations in genes such as *BMP1, FKBP10* were reported in their cohort of 50 patients. This highlights the utility of gene panel testing in a setting where specific mutations are known to be more common. WES can be particularly useful in cases where patients present with atypical features or where the targeted gene panel does not provide a definitive result. For example, in one patient, WES revealed no significant mutations, suggesting the presence of non-coding or complex inter-related genetic contributions to the disease that may have been missed by targeted panels. Targeted sequence analysis is often more practical and cost-effective when a patient's clinical presentation strongly points to mutations in known OI-associated genes. WES is more comprehensive and can reveal unexpected mutations, but is also more resource intensive. The choice between these techniques may depend on clinical indications, resources and the possibility of atypical genetic contribution to the disease (53).

Study Limitations

There are some limitations to the present study due to the small size of the study population, single-center nature, and possible selection bias due to being only one tertiary center. Furthermore, the cases' pedigrees were not considered because the study was conducted a long time ago. The exclusion of certain genes associated with osteogenesis imperfecta *(CCDC134, CREB3L1, KDELR2, MESD, SPARC, TENT5A, TNEN38B, WNT1)* also contributes to the study's limitations.

Conclusion

This fairly comprehensive study demonstrated the clinical and molecular features of OI in a clinically diagnosed Turkish cohort. Genetic etiology was identified in 82.6% of 46 families with targeted NGS analysis. In addition, nine novel variants in OI-associated genes were identified. However, no new candidate gene related to OI could be detected by NGS analysis and eight genes known to be associated with OI were not analyzed (see limitations above). In patients where variants cannot be detected by targeted NGS, advanced genetic analysis, such as WES analysis may be planned. Finally, targetted panel studies in genetically heterogeneous diseases like OI are helpful for increasing the rate of variant detection.

Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of the Ege University Medical Faculty (ethic committee number: 18-3.1/55, date: 20.03.2018), and samples from the patients were obtained in accordance with the Helsinki Declarations.

Informed Consent: Written informed consent for molecular analysis was obtained from all cases or their parents/guardians.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ferda Evin, Tahir Atik, Şükran Darcan, Özgür Çoğulu, Concept: Samim Özen, Design: Samim Özen, Damla Gökşen, Data Collection or Processing: Esra Işık, Hüseyin Onay, Bilçağ Akgün, Aysun Ata, Füsun Düzcan, Şükran Darcan, Özgür Çoğulu, Analysis or Interpretation: Hüseyin Onay, Bilçağ Akgün, Aysun Ata, Füsun Düzcan, Şükran Darcan, Özgür Çoğulu, Literature Search: Esra Işık, Hüseyin Onay, Tahir Atik, Ferda Özkınay, Writing: Damla Gökşen, Ferda Evin, Bilçağ Akgün, Aysun Ata, Füsun Düzcan.

Conflict of Interest: Two authors of this article, Samim Özen and Damla Gökşen, are a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, they were not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

Financial Disclosure: This project was supported by Ege University Scientific Research Projects with grant number: TGA-2018-20185.

References

1. Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. Nat Rev Endocrinol. 2011;7:540-557.
2. Rossi V, Lee B, Marom R. Osteogenesis imperfecta: advancements in genetics and treatment. Curr Opin Pediatr. 2019;31:708-715.
3. Palomo T, Vilaça T, Lazaretti-Castro M. Osteogenesis imperfecta: diagnosis and treatment. Curr Opin Endocrinol Diabetes Obes. 2017;24:381-388.
4. Erbaş İM, İlgün Gürel D, Manav Kabayeğit Z, Koç A, Ünüvar T, Abacı A, Böber E, Anık A. Clinical, genetic characteristics and treatment outcomes of children and adolescents with osteogenesis imperfecta: a two-center experience. Connect Tissue Res. 2022;63:349-358. Epub 2021 Jun 9
5. Gupta N, Gregory SW, Deyle DR, Tebben PJ. Three Patient Kindred with a Novel Phenotype of Osteogenesis Imperfecta due to a COL1A1 Variant. J Clin Res Pediatr Endocrinol. 2021;13:218-224. Epub 2020 Jun 10
6. Marini JC, Forlino A, Bächinger HP, Bishop NJ, Byers PH, Paepe A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. Nat Rev Dis Primers. 2017;3:17052.
7. Marini JC, Blissett AR. New genes in bone development: what's new in osteogenesis imperfecta. J Clin Endocrinol Metab. 2013;98:3095-3103. Epub 2013 Jun 14
8. Forlino A, Marini JC. Osteogenesis imperfecta review 2015, The Lancet 2015;363:1377-1385.
9. Patel R, Camacho P. Osteogenesis imperfecta: Case-Based Approach. Metab Bone Dis. 2019;123-135.
10. Morello R. Osteogenesis imperfecta and therapeutics. Matrix Biol. 2018;71-72:294-312. Epub 2018 Mar 11
11. Forlino A, Marini JC. Osteogenesis imperfecta. The Lancet. 2016;387:1657-1671.
12. Demir K, Konakçı E, Özkaya G, Kasap Demir B, Özen S, Aydın M, Darendeliler F. New Features for Child Metrics: Further Growth References and Blood Pressure Calculations. J Clin Res Pediatr Endocrinol. 2019;12:125-129. Epub 2019 Sep 2
13. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015;7:280-293.
14. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013:7.
15. Goode DL, Cooper GM, Schmutz J, Dickson M, Gonzales E, Tsai M, Karra K, Davydov E, Batzoglou S, Myers RM, Sidow A. Evolutionary constraint facilitates interpretation of genetic variation in resequenced human genomes. Genome Res. 2010;20:301-310. Epub 2010 Jan 12
16. Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 2010;20:110-121. Epub 2009 Oct 26
17. J Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010;7:575-576.
18. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-423. Epub 2015 Mar 5
19. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014;164:1470-1481. Epub 2014 Apr 8
20. Loman NJ, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ. Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol. 2012;30:434-439.
21. van Dijk FS, Huizer M, Kariminejad A, Marcelis CL, Plomp AS, Terhal PA, Meijers-Heijboer H, Weiss MM, van Rijn RR, Cobben JM, Pals G. Complete COL1A1 allele deletions in osteogenesis imperfecta. Genet Med. 2010;12:736-741.
22. Stephen J, Girisha KM, Dalal A, Shukla A, Shah H, Srivastava P, Kornak U, Phadke SR. Mutations in patients with osteogenesis imperfecta from consanguineous Indian families. Eur J Med Genet. 2015;58:21-27. Epub 2014 Oct 24
23. Maioli M, Gnoli M, Boarini M, Tremosini M, Zambrano A, Pedrini E, Mordenti M, Corsini S, D'Eufemia P, Versacci P, Celli M, Sangiorgi L. Genotype-phenotype correlation study in 364 osteogenesis imperfecta Italian patients. Eur J Hum Genet. 2019;27:1090-1100. Epub 2019 Mar 18
24. Lee KS, Song HR, Cho TJ, Kim HJ, Lee TM, Jin HS, Park HY, Kang S, Jung SC, Koo SK. Mutational spectrum of type I collagen genes in Korean patients with osteogenesis imperfecta. Hum Mutat. 2006;27:599.
25. Lin HY, Chuang CK, Su YN, Chen MR, Chiu HC, Niu DM, Lin SP. Genotype and phenotype analysis of Taiwanese patients with osteogenesis imperfecta. Orphanet J Rare Dis. 2015;10:152.

26. Venturi G, Tedeschi E, Mottes M, Valli M, Camilot M, Viglio S, Antoniazzi F, Tatò L. Osteogenesis imperfecta: clinical, biochemical and molecular findings. Clin Genet. 2006;70:131-139.

27. Zhang ZL, Zhang H, Ke YH, Yue H, Xiao WJ, Yu JB, Gu JM, Hu WW, Wang C, He JW, Fu WZ. The identification of novel mutations in COL1A1, COL1A2, and LEPRE1 genes in Chinese patients with osteogenesis imperfecta. J Bone Miner Metab. 2012;30:69-77. Epub 2011 Jun 14

28. Bregou Bourgeois A, Aubry-Rozier B, Bonafé L, Laurent-Applegate L, Pioletti DP, Zambelli PY. Osteogenesis imperfecta: from diagnosis and multidisciplinary treatment to future perspectives. Swiss Med Wkly. 2016;146:w14322.

29. Tournis S, Dede AD. Osteogenesis imperfecta - A clinical update. Metabolism. 2018;80:27-37.

30. Mohd Nawawi N, Selveindran NM, Rasat R, Chow YP, Abdul Latiff Z, Syed Zakaria SZ, Jamal R, Abdul Murad NA, Abd Aziz BB. Genotype-phenotype correlation among Malaysian patients with osteogenesis imperfecta. Clin Chim Acta. 2018;484:141-147. Epub 2018 May 25

31. Hald JD, Folkestad L, Harsløf T, Lund AM, Duno M, Jensen JB, Neghabat S, Brixen K, Langdahl B. Skeletal phenotypes in adult patients with osteogenesis imperfecta-correlations with COL1A1/COL1A2 genotype and collagen structure. Osteoporos Int. 2016;27:3331-3341. Epub 2016 Jun 2

32. Saeves R, Lande Wekre L, Ambjørnsen E, Axelsson S, Nordgarden H, Storhaug K. Oral findings in adults with osteogenesis imperfecta. Spec Care Dentist. 2009;29:102-108.

33. Andersson K, Dahllöf G, Lindahl K, Kindmark A, Grigelioniene G, Åström E, Malmgren B. Mutations in COL1A1 and COL1A2 and dental aberrations in children and adolescents with osteogenesis imperfecta - A retrospective cohort study. PLoS One. 2017;12:e0176466.

34. Lindahl K, Åström E, Rubin CJ, Grigelioniene G, Malmgren B, Ljunggren Ö, Kindmark A. Genetic epidemiology, prevalence, and genotype-phenotype correlations in the Swedish population with osteogenesis imperfecta. Eur J Hum Genet. 2015;23:1042-1050. Epub 2015 May 6

35. Malmgren B, Andersson K, Lindahl K, Kindmark A, Grigelioniene G, Zachariadis V, Dahllöf G, Åström E. Tooth agenesis in osteogenesis imperfecta related to mutations in the collagen type I genes. Oral Dis. 2017;23:42-49. Epub 2016 Sep 13

36. Abali S, Arman A, Atay Z, Bereket A, Bas S, Haliloglu B, Guran T, Gormez Z, Demirci H, Akarsu N, Turan S. Frequency of Recessive Osteogenesis Imperfecta in a Turkish Cohort and Genetic Causes. 55th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE), Paris, France, September 10-12, 2016: Abstracts, Horm Res Paediatr. 2016;86(Suppl 1):175-176.

37. Ho Duy B, Zhytnik L, Maasalu K, Kändla I, Prans E, Reimann E, Märtson A, Kõks S. Mutation analysis of the COL1A1 and COL1A2 genes in Vietnamese patients with osteogenesis imperfecta. Hum Genomics. 2016;0:27.

38. Patel RM, Nagamani SC, Cuthbertson D, Campeau PM, Krischer JP, Shapiro JR, Steiner RD, Smith PA, Bober MB, Byers PH, Pepin M, Durigova M, Glorieux FH, Rauch F, Lee BH, Hart T, Sutton VR. A cross-sectional multicenter study of osteogenesis imperfecta in North America - results from the linked clinical research centers. Clin Genet. 2015;87:133-140. Epub 2014 May 30

39. Ji Y, Ikram A, Ma Z, Peppelenbosch MP, Pan Q. Co-occurrence of heterozygous mutations in COL1A1 and SERPINF1 in a high-risk pregnancy complicated by osteogenesis imperfecta. J Genet. 2019;98:51.

40. Cabral WA, Barnes AM, Adeyemo A, Cushing K, Chitayat D, Porter FD, Panny SR, Gulamali-Majid F, Tishkoff SA, Rebbeck TR, Gueye SM, Bailey-Wilson JE, Brody LC, Rotimi CN, Marini JC. A founder mutation in LEPRE1 carried by 1.5% of West Africans and 0.4% of African Americans causes lethal recessive osteogenesis imperfecta. Genet Med. 2012;14:543-551. Epub 2012 Jan 26

41. Baldridge D, Schwarze U, Morello R, Lennington J, Bertin TK, Pace JM, Pepin MG, Weis M, Eyre DR, Walsh J, Lambert D, Green A, Robinson H, Michelson M, Houge G, Lindman C, Martin J, Ward J, Lemyre E, Mitchell JJ, Krakow D, Rimoin DL, Cohn DH, Byers PH, Lee B. CRTAP and LEPRE1 mutations in recessive osteogenesis imperfecta. Hum Mutat. 2008;29:1435-1442.

42. Pepin MG, Schwarze U, Singh V, Romana M, Jones-Lecointe A, Byers PH. Allelic background of LEPRE1 mutations that cause recessive forms of osteogenesis imperfecta in different populations. Mol Genet Genomic Med. 2013;1:194-205. Epub 2013 Jun 26

43. Bardai G, Moffatt P, Glorieux FH, Rauch F. DNA sequence analysis in 598 individuals with a clinical diagnosis of osteogenesis imperfecta: diagnostic yield and mutation spectrum. Osteoporos Int. 2016;27:3607-3613. Epub 2016 Aug 11

44. Al-Jallad H, Palomo T, Roughley P, Glorieux FH, McKee MD, Moffatt P, Rauch F. The effect of SERPINF1 in-frame mutations in osteogenesis imperfecta type VI. Bone. 2015;76:115-120. Epub 2015 Apr 11

45. Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C. Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. Am J Hum Genet. 2011;88:362-371. Epub 2011 Feb 25

46. Rauch F, Husseini A, Roughley P, Glorieux FH, Moffatt P. Lack of circulating pigment epithelium-derived factor is a marker of osteogenesis imperfecta type VI. J Clin Endocrinol Metab. 2012;97:1550-1556. Epub 2012 Jun 5

47. Shaheen R, Al-Owain M, Sakati N, Alzayed ZS, Alkuraya FS. FKBP10 and Bruck syndrome: phenotypic heterogeneity or call for reclassification? Am J Hum Genet. 2010;87:306-307.

48. Alanay Y, Krakow D. Response to Shaheen et al. Am J Hum Genet. 2010;87:306-308.

49. Rauch F, Lalic L, Roughley P, Glorieux FH. Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. J Bone Miner Res. 2010;25:1367-1374.

50. Ben Amor IM, Glorieux FH, Rauch F. Genotype-phenotype correlations in autosomal dominant osteogenesis imperfecta. J Osteoporos. 2011;2011:540178. Epub 2011 Sep 6

51. Zhytnik L, Maasalu K, Pashenko A, Khmyzov S, Reimann E, Prans E, Kõks S, Märtson A. COL1A1/2 Pathogenic Variants and Phenotype Characteristics in Ukrainian Osteogenesis Imperfecta Patients. Front Genet. 2019;10:722.

52. Mrosk J, Bhavani GS, Shah H, Hecht J, Krüger U, Shukla A, Kornak U, Girisha KM. Diagnostic strategies and genotype-phenotype correlation in a large Indian cohort of osteogenesis imperfecta. Bone. 2018;110:368-377. Epub 2018 Feb 27

53. Lim J, Grafe I, Alexander S, Lee B. Genetic causes and mechanisms of Osteogenesis Imperfecta. Bone. 2017;102:40-49. Epub 2017 Feb 15