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Research Article

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

Özen S et al. Molecular Genetic Diagnosis in a Cohort of Osteogenesis Imperfecta

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 Medical Faculty Department of Pediatrics, Subdivision of Pediatric Endocrinology, Izmir, Turkey
[2] Department of Pediatric Endocrinology, Bakırçay University Çiğli Training and Research Hospital, Izmir, Turkey
[3] Ege University Medical Faculty Department of Pediatrics, Subdivision of Pediatric Genetics, Izmir, Turkey
[4] Ege University Medical Faculty Department of Medical Genetics, Izmir, Turkey
[5]Tınaztepe University Medical Faculty Department of Medical Genetics, Izmir, Turkey

What is already known on this topic?
Variants in *COL1A1* and *COL1A2* genes encoding type I collagen are responsible for most of the etiology. 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 osteogenesis imperfecta 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 and we think that it was a valuable method for genetic diagnosing of this patients.

Ferda Evin MD, Department of Pediatric Endocrinology, Bakırçay University Çiğli Training and Research Hospital, Izmir, Turkey
https://orcid.org/0000-0001-7169-890X
ferdaevin88@gmail.com
+90-553-307-35-90
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Abstract
Introduction: Osteogenesis imperfecta (OI) is a group of phenotypically and genetically heterogeneous connective tissue disorders that share similar skeletal anomalies causing bone fragility and deformation. This study aimed to investigate the molecular genetic etiology and determine the relationship between genotype and phenotype in OI patients with targeted next-generation sequencing (NGS).
Method: In patients with OI, a targeted NGS analysis panel (Illumina TruSight One) containing genes involved in collagen/bone synthesis was performed on the Illumina Nextseq550 platform.
Results: Fifty-six patients (female/male: 25/31) from 46 different families were enrolled in the study. Consanguinity between parents was noted in 15 (32.6%) families. Clinically according to Sillence classification; 18(33.1%) patients were considered to type I, 1(1.7%) type II, 26(46.4%) type III and 11(19.6%) type IV. Median body weight was -1.1 (-6.8, - 2.5) SDS, and height was -2.3 (-7.6, - 1.2) SDS. 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 dentinogenesis imperfecta (DI), and 2 (3.6%) had hearing loss. Disease-causing variants in *COL1A1* and *COL1A2* genes 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 variants in three different genes (*FKBP10, SERPINF1*, and *P3H1*). Nine (23.6%) of the variants detected in all investigated genes were not previously reported in the literature and were classified to be pathogenic according to *ACMG* guidelines pathogenity scores. In ten (21.7%) families, a disease-related variant was not found in a total of 13 OI genes included in the panel.
Conclusion: Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis. In addition, 9 new variants were assessed in known OI genes which is a significant contribution to the literature.
Keywords: Osteogenesis imperfecta, next-generation sequencing, COL1A1, genetics

1. Introduction
Osteogenesis imperfecta (OI) is a hereditary disease of connective tissue characterized by increased bone fragility and multiple fractures [1–3]. OI is a rare disorder with a frequency of 1/15.000-20.000. This generalized connective tissue disorder has important signs in the bone, leading to skeletal fragility and developmental delay. It has also different clinical severity and additional features such as dentinogenesis imperfecta, blue sclerae, short stature, hearing loss, and cardiac malformations [4]. Variants in the collagen genes are responsible for approximately 85-90% of OI. The patients with OI have heterogeneous clinical and genetic features [5]. Therefore, clinical diagnosis is insufficient for diagnosis, management, prognosis, and genetic counseling. In recent years, new OI types have been discovered with the development of genetic analysis techniques. The disorder can also be caused by variants of genes related to collagen structure and function [6–8]. The most recently identified genes today are characterized by primary defects in osteoblast differentiation [2,3,6,9].
Advances in next-generation sequencing (NGS) technology have enabled the discovery of novel genes and pathogenic variants related to OI [2,10,11]. Molecular diagnosis is useful for early diagnosis, estimating the prognosis, determining other individuals in the family, and choosing treatment according to knowledge of variable responses to drugs [2,10–12].
In this study, we aimed 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.

2. Method
2.1 Study group

Clinically and/or radiologically diagnosed 56 OI patients from 46 different families followed in Ege University Faculty of Medicine Pediatric Endocrinology Clinic 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 (steroids, chemotherapy, etc.) were excluded.
Demographical 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 scores (SDS) were calculated based on Turkish standards [13,14]. The study was approved by the Ethical Committee of the Ege University Medical Faculty (Ethic Committee Number: 16-2.1/16), 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.

2.2 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)* responsible for OI was used.

2.3 Data analysis

Sequencing data was analyzed using Illumina VariantStudio software and IGV (Integrative Genomics Viewer). Firstly, 13 genes known to be responsible for the 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 (ESP) 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 [15–18]. The pathogenicity of all variants identified was classified according to the American College of Medical Genetics (ACMG) recommendations. The pathogenity scores were searched from https://www.acmg.net/ website. The ACMG Guidelines were established by clinicians and clinical lab directors who are experts in clinical genetics and part of the American College of Medical Genetics and Genomics (ACMG), the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP). 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 (LB), and Benign (B), depending on the applied criteria. These criteria can be classified by the weight and type of evidence indicated by each criterion. The 28 criteria can be classified into 8 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 [19]. Secondly, patients found to have no variant in the OI genes were then analyzed for all other genes included in the panel.

2.4 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).

3. Results

3.1 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 patients 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 [20] (Table 1), 18 (33.1%) patients were considered to be type I, 1 (1.7%) type II, 26 (46.4%) type III, and 11 (19.6%) type IV. 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 dentinogenesis imperfecta (DI), and 2 (3.6%) had hearing loss.

3.2 Molecular analysis findings

Sequence analysis of *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 the *COL1A2* gene in one patient.The variant detected in the *COL1A1* gene was also found in the asymptomatic father of the patient in the segregation analysis. According to this result, 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 detected variants in all genes have not been previously reported in the literature 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.

3.3 Genotype-phenotype relations

Fifteen of the *COL1A1* variants were boys, and 14 were girls. The mean age at the 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 patients (44.8%) Type 1, 1 (3.4%) Type II, 10 (34.4%) Type III, and 5 (17.2%) Type IV. Recurrent pathological fractures 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, 2 boys) 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 III and 2 (33.3%) IV. In all patients, 2 or more recurrent pathological bone fractures and deformities were detected. In 5 (50%) patients, blue sclera and in one (16.6%) patient, DI was detected.

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 III with severe deformities, recurrent fractures, and short stature. However, family segregation was not performed on 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 Type IV clinic. Her parents were heterozygous for the same variant. The same variant was demonstrated in the case's sibling with a similar phenotype. These cases had severe osteogenesis imperfecta and congenital contractures of large joints, short stature, and scoliosis.

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

4. Discussion

In this study, we aimed to determine the molecular etiology of 56 clinically diagnosed OI patients from 46 different families. The NGS panel (covering a total of 4800 genes, including 13 genes related to OI, was applied. Genetic etiology was found in 38 (82.6%) of 46 families by targeted NGS analysis with 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 now called “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, the “clinical exome” only contains genes associated with the disease in humans. The advantage of TruSight One panel compared to WES is that it is easier to analyze the results, and the cost is lower [21].

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 protein is not rapidly degraded but instead when it is incorporated in the triple helix exerting a dominant negative effect. In a study by van Dijk et al., multiplex ligation-dependent probe amplification analysis was performed in the analysis of the *COL1A1* gene in a group of 106 index patients. They found 7 patients with deletion of the complete *COL1A1* gene on one allele [22]. In our study, we did not evaluate the gross deletions and duplications in exon because this is outside the scope of the analysis methods. Although identified in very small rates in general, it may be more relevant for our group without any variants regarding the disease severity via haploinsufficiency of *COL1A1* and *COL1A2* genes.

Consanguineous marriage was revealed in 28.5% of patients, and 39.3% had an OI family history. Consanguineous marriage may lead to a high rate of AR variants to be found. In a study in India with 7 patients of consanguineous marriage, *SERPINF1, PPIB*, and *CRTAB* mutations detected [23]. In a study evaluating *COL1A1* and *COL1A2* gene variants of 364 patients of Italian origin, family history rate was reported as 57.7% [24]. However, in this study, the rate may be high since other types of OI other than these two OD-inherited genes were not studied. In the literature, family history rates were reported as 53% in the Korean population [25], and as 32-33% in different societies [26–28]. The reason for this difference may be due to genetic differences in societies, variations in the genetic analysis method of studies, and the changing frequency of de novo variants. Again, according to the frequency of consanguineous marriage and founder variant in society, the distribution of genes responsible for OI can be variable.

The blue sclera is one of the distinctive clinical features of OI and is frequently observed in OI type I patients. Type III and IV OI patients may have blue sclera at birth, but the bluish color disappears with increasing age [2,9,29]. In our study group, 34 (60.7%) of the patients had blue sclera.

Bone fractures and deformities in OI usually occur at an early age and are often caused by repeated bone remodeling in long bones. [2,30]. 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. Nawawi et al., [31] showed that 63.6% of all OI patients had bone deformities at the age of 9 and had to walk with help. Studies have shown that bone deformities are more common in patients with qualitative variants than quantitative variants. [26,31]. Hald et al. [32] showed that OI patients with quantitative defects had normal protein structure in bone despite collagen deficiency. This allows bone mineralization and only causes less breakage than qualitative defects [12,32].

Dentinogenesis imperfecta has been reported in type III and less, especially in type I OI. [33,34]. In our study, DI was detected in 12 (21.4%) of the patients. The clinical diagnosis distribution of the patients with dentinogenesis imperfecta was as follows: 5 patients (41.6%) were Type I, 4 (33.3%) Type III, and 3 (25%) Type IV. In another study, DI was reported to be more frequent in patients with more severe clinical types (type III and IV) than in moderate groups (type I). [35]. Those with a qualitative variant (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. [36].

In our study, 63.1% had a variant in the *COL1A1* gene, 13.1% in the *COL1A2* gene, and 2.6% in both genes; totally of 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, 3 different genes (*SERPINF1, FKBP10*, and *P3H1)* variants were detected. 3 (7.8%) families had *FKBP10*, 3 (7.8%) families had *SERPINF1,* and 2 (5.2%) families had the *P3H1* variant. Abalı et al. [37] studied 89 patients with OI. Similar to our study, 61.4% had variants in *COL1A1* and *COL1A2* genes, 5 (5.6%) patients had *FKBP10*, 2 (2.2%) *LRP5*, 1 (1.1%) *P3H1*, 1 (1.1%) *CRTAP*, 1 (1.1%) *BMP1* and 1 (1.1%) *SPARC* genes variants.

COL1A1 and COL1A2 Gene Variants

Variants in *COL1A1* and *COL1A2* genes encoding type I collagen are responsible for most of the etiology. In our study, 62.4% of patients had variants in these two genes. In previous studies, variants in these two genes were responsible for 51-73% of the disease. [26,31,38,39]. Similar to other studies, variants in the COL1A1 gene were detected more frequently than the *COL1A2* gene. [26,28,31,40].

In one (3.3%) case (Patient 7), the 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 the segregation analysis. According to this result, it was thought that the VUS variant in the *COL1A1* gene did not cause 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. In the literature, no cases carrying variants of these two genes at the same time have been reported. However, JiY et al. reported a case with a severe clinic variant in *COL1A1* and *SERPINF1* genes[41]. 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 is %0,05 and %0,02 in 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 for association with Osteogenesis imperfecta (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 2 months of age. This variant was thought to be disease-causing *in silico* analysis and was not previously identified. Recurrent fractures continued with severe clinical phenotypes. In the literature, clinical types of 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. This variant was thought to be the founding variant [42–44]. This carriage was not observed in our 56 patients.

SERPINF1 Gene Variants

In some populations, *SERPINF1* and *CRTAP* variants were responsible for recessive OI types. They even reported some variants causing a "founder" effect [45].

In our study, 4 different variants were detected in 3 patients from different families, and another variant was detected in 2 siblings. Patients with *SERPINF1* variants had a heavier 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 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 our patients, in the literature, most *SERPINF1* gene variants have been reported to be caused by frameshift and nonsense variants. [46,47]. 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 is predicted to be VUS by Franklin Genoox and Varsome programmes according to ACMG 2015 criteria. Most of the predictions tool predicted that this variant will be pathogenic or VUS. This variant has low population frequency. With these evidences it was thought that homozygous c.317G>C p.(Arg106Pro) variant detected in SERPINF1 gene might be responsible for the clinical picture of the patient. These variants impair circulating pigment epithelium-derived factor (PEDF) production as well as loss of PEDF protein function [2]. Rauch et al. [48] 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 [2,49]. In 2 brothers with Bruck syndrome, Shaheen et al. [50] reported a homozygous 8-bp insertion variant in the *FKBP10* gene. Alanay et al. [51] reported that the patients in Shaheen et al.'s study may have Bruck syndrome and that the clinic 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 Osteogenesis Imperfecta have been extensively studied over the years, with certain investigations revealing significant associations [52,53]. Notably, more severe phenotypes have been observed in patients harboring pathogenic variants in *COL1A1* compared to those in *COL1A2* [54]. Mrosk et al. asserted 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 [55]. In our 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. Additionally, we identified a variant in a total of 15 affected individuals across 7 families. Remarkably, the clinical types and features of cases with the same variant within these families were notably 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 cases 11 and 12 was similar. However, while case 41 was type 4 OI, case 41 was type 3 OI. 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 [56,57]. The genotype-phenotype relationship in osteogenesis imperfecta remains to be determined, as carriers of the same variant may develop diverse phenotypes. Furthermore, the factors influencing additional phenotype modifications have yet to be fully elucidated.

In our 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 known genes or 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 has 82% diagnostic yield. For most of the cases this high diagnostic yield is fascinating. But today's technology allows us to perform WES-CNV at a comparable price. But in most of the institutions the capacity of the genetic laboratories is the main determinant of the genetic approach.

In one study, they noted that an unusually high percentage of autosomal recessive forms due to mutations in genes such as *BMP1, FKBP10* and others were discovered 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.

Whole exome sequencing 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 in the study, WES revealed no significant mutations, suggesting the presence of non-coding or complex 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. Whole exome sequencing 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 [58].

Limitations: There are some limitations to the present study due to the small size of the study population, single-center, and possible selection bias is possible due to one tertiary center. Further, it did not access the cases' pedigrees 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.

5. Conclusion

This comprehensive study demonstrates the clinical and molecular features of OI disease. Genetic etiology was determined in 38 (82.6%) of 46 families with the targeted NGS analysis. In addition, nine variants in OI genes have been identified, making an important contribution to the literature. However, in this study, no new candidate gene related to OI could be detected by NGS analysis. In patients where variants cannot be detected, advanced genetic analysis, such as whole exome sequence analysis will be planned. Panel studies in such genetically heterogeneous diseases are critical for increasing the rate of variant detection.

Ethics approval: The study was approved by the Ethical Committee of the Ege University Medical Faculty (Ethic Committee Number: 16-2.1/16), 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.

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Table 1: The actualized Sillence classification*

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

* Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014;164A:1470–81

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 III	Mobile	3	Yes, lower extremity	+	-	-	1/F	8,3
COL1A1	c.1283delG (p.Gly428Valfs*113)	No	Type I	Mobile	3	No	+	-	-	2/F	1,8
			Type I	Mobile	No	No	+	-	-	3/F	1,2
			Type I	Mobile	3	No	+	-	-	4/M	6,7
COL1A1	c.1699C>T (p.Pro567Ser)	No	Type IV	Mobile	3	Yes, lower extremity	+	-	+	5/M	2,4
			Type IV	Mobile	No	No	+	-	+	6/M	6,1
COL1A1 *COL1A2*	c.3677A>G (p.Asp1226Gly)/ c.2296G>C (p.Gly766Arg)	Yes	Type IV	Mobile	2	Yes, lower extremity	+	-	-	7/F	2.5 months
COL1A1	c.626G>A (p.Gly209Asp)	Unknown	Type III	Assisted walking	3	Yes, very severe	+	-	-	8/M	8.5 months
COL1A1	c.1057-2A>C	Yes	Type III	Mobile	6	No	+	-	-	9/M	3,7
COL1A1	c.1081C>T (p.Arg361*)	No	Type I	Mobile	3	No	+	-	-	10/F	3,6
COL1A1	c.1299+1G>A	No	Type I	Mobile	3	No	+	-	-	11/M	12,8
COL1A1	c.1299+1G>T	No	Type I	Mobile	4	Yes, lower extremity	+	-	-	12/M	7,9
COL1A1	c.1353+2T>C	No	Type III	Assisted walking	2	Yes, lower extremity	+	-	-	13/F	1.5 months

COL1A1	c.1405C>T (p.Arg469*)	No	Type I	Mobile	2	No	+	-	+	14/F	3,4
COL1A1	c.2596G>A (p.Gly866Ser)	No	Type II	Sitting	1	No	+	-	-	15/F	1.5 months
COL1A1	c.3235G>A (p.Gly1079Ser)	No	Type III	Mobile	3	No	+	-	-	16/M	7,8
			Type III	Mobile	3	No	+	-	-	17/M	10 months
COL1A1	c.3505G>A (p.Gly1169Ser)	No	Type III	Assisted walking	3	Yes, lower extremity	+	-	-	18/F	10,6
COL1A1	c.1128delT (p.Gly377Alafs*164)	No	Type I	Mobile	2	No	+	-	-	19/F	3,0
			Type I	Mobile	3	No	+	-	-	20/M	2,9
COL1A1	c.1459_1460insA (p.Arg487Glnfs*6)	No	Type I	Mobile	2	No	+	-	+	21/F	8.5 months
COL1A1	c.958G>C (p.Gly320Arg)	No	Type IV	Mobile	3	No	-	-	-	22/M	9,5
COL1A1	c.4051C>T (p.Gln1351*)	No	Type III	Mobile	4	No	-	-	-	23/M	10,2
COL1A1	c.441delC (p.Gly148Aspfs*117)	No	Type I	Mobile	3	No	+	-	+	24/M	7,1
COL1A1	c.886G>T (p.Gly296*)	No	Type IV	Mobile	2	No	+	-	-	25/F	3,9
COL1A1	c.1156-1G>A	Yes	Type I	Mobile	1	No	+	-	-	26/F	4,6
COL1A1	c.3647A>G (p.Tyr1216Cys)	No	Type IV	Mobile	2	No	-	-	-	27/F	2,7
COL1A1	c.608G>T (p.Gly203Val)	No	Type III	Sitting	5	Yes, lower extremity	+	-	+	28/M	1,7
COL1A1	c.1405C>T (p.Arg469*)	No	Type I	Mobile	5	No	+	-	+	29/M	9,7
COL1A1	c.2829+2dupT	No	Type III	Mobile	2	No	+	-	-	30/F	1,1
COL1A2	c.1972G>A (p.Gly658Ser)	No	Type III	Assisted walking	4	Yes, lower extremity	-	-	-	31/F	10,3
COL1A2	c.3250G>T (p.Gly1084Cys)	No	Type IV	Assisted walking	2	Yes, lower extremity	+	-	-	32/M	10 months
COL1A2	c.928G>C (p.Gly310Arg)	Yes	Type III	Assisted sitting	3	Yes, lower and upper extremity	+	-	+	33/M	3.5 months
COL1A2	c.1081G>A (p.Gly361Ser)	No	Type III	Assisted walking	3	Yes, spine	-	-	-	34/F	5,7
COL1A2	c.3014G>A (p.Arg1005His)	No	Type III	Immobile	3	Yes, very severe	-	+	-	35/F	6,5
SERPINF1	c.80dupA (p.Glu28Glyfs*37)/ c.907C>T (p.Arg303*)	No	Type III	Immobile	4	Yes, very severe	-	-	-	36/F	8.5 months
SERPINF1	c.317G>C (p.Arg106Pro)	Yes	Type I	Mobile	2	No	+	-	-	37/F	8,6
SERPINF1	c.988C>T (p.Gln330*)	Yes	Type I	Mobile	2	No	+	-	-	38/M	11,6
			Type III	Assisted sitting	4	Yes, spine	-	-	-	39/F	6 months
P3H1	c.446T>G (p.Leu149Arg)	Yes	Type IV	Mobile	4	Yes, lower extremity	-	-	-	40/F	2.5 months

P3H1	c.446T>G (p.Leu149Arg)	Yes	Type III	Assisted walking	3	Yes, lower and upper extremity	-	-	-	41/F	1.5 months
FKBP10	c.1490G>A (p.Trp497*)	No	Type IV	Assisted walking	4	Yes, lower and upper extremity	-	-	-	42/M	4,3
			Type IV	Sitting	3	Yes, lower and upper extremity	-	-	-	43/M	5 months
FKBP10	c.831dupC (p.Gly278Argfs*95)	Yes	Type III	Assisted sitting	3	Yes, lower and upper extremity	-	-	+	44/M	4,6
FKBP10	c.21dupC (p.Ser8Glnfs*67)	No	Type III	Immobile	3	Yes, very severe	-	-	-	45/F	8,3
			Type III	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

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 *COL1A2*	c.3677A>G (p.Asp1226Gly)/ c.2296G>C (p.Gly766Arg)	NM_000088.4 NM_000089.4	chr17-48264138 chr7-94050321	rs1319157667	VUS P	N/A	0.0032
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
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 chr17-1679946	 rs763291398	LP P	0.0025	0.0046
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

LP: Likely Pathogenic, VUS: Variant of Unknown Significance, P: Pathogenic
* Exome Aggregation Consortium (http://exac.broadinstitude.org),
#The allele frequency in the ExAC database does not represent all ethnic groups