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Case Report

A Rare Pathogenic Variant in the *SERPINF1* Gene in Association with Early-Onset Severe Presentation of Autosomal Recessive Type of Osteogenesis Imperfecta VI: A Case Report

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Keywords

Autosomal recessive · Osteogenesis imperfecta · Pathogenic variant · Rare mutation · SERPINF1 · Truncating mutation

Abstract

Osteogenesis imperfecta (OI) refers to a group of genetic disorders with the typical clinical presentation of increased bone fragility. More than 90% of OI cases have an underlying genetic mutation in the collagen genes *COL1A1* or *COL1A2*. However, there are now 22 different recognized OI subtypes based on the underlying gene implicated. OI type VI is caused by pathogenic variants in the serine proteinase inhibitor clade F (*SERPINF1*) gene. In this case study, the patient was a 4-year-old female, born in a consanguineous family, with a history of delayed gross motor milestones and recurrent fractures after trivial trauma since the age of 8 months. The results of skeletal surveys and bone scans indicated that the patient suffered from OI. To confirm the diagnosis, we performed a next-generation sequencing (NGS) analysis of genes implicated in OI using the TruSight One NGS Panel. Bioinformatics analysis showed the presence of a rare pathogenic variant in exon 7 of the *SERPINF1* gene in a homozygous state (NM_002615.5:c.907C>T; NP_002606.3:p.Arg303Ter; ClinVar submission ID: SUB10491966). The variant results in the termination of the polypeptide chain at position 303. Targeted capillary sequencing confirmed the presence of this variant in a homozygous state in the patient and in heterozygous state for both parents. Additionally, the patient's younger sibling, aged

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10 months, harbored the variant in a homozygous state. The sibling started bisphosphonate therapy and did not suffer any fractures in over 2 years after initiating the therapy. The etiological genetic diagnosis helped provide genetic counseling to the family. This highlights the utility of NGS for the confirmation of a clinical analysis in cases where alterations in multiple genes can result in similar clinical presentation.

Introduction

Osteogenesis imperfecta (OI; MIMs 166200, 166210, 259420, 166220, 610967, 613982, 610682, 610915, 259440, 613848, 610968, 613849, 614856, 615066, 615220, 616229, 616507, 617952, 301014, 618644, 619131, and 619795 for types I to XXII OI, respectively) is a group of genetic disorders that exhibit a common clinical presentation, i.e., bone fragility. OI is mainly caused by abnormalities in type I collagen, which leads to a generalized connective tissue disorder. More than 90% of individuals affected with OI harbor pathogenic variants in the *COL1A1* or *COL1A2* genes associated with an autosomal dominant mode of inheritance. In the early 2000s, it was discovered that OI can be caused by a plethora of genes other than *COL1A1* and *COL1A2* [1–7]; the original Sillence classification has now been expanded to include twenty-two OI subtypes based on genetic classification [8]. Biallelic pathogenic/likely pathogenic (P/LP) variants in the serine proteinase inhibitor clade F gene (*SERPINF1*) were first reported in 2002 in 6 patients with early-onset severe presentation of OI (type VI OI). The individuals had more severe disease presentation and the absence of dentinogenesis imperfecta [4]. Here, we report a case of early-onset severe OI with parental consanguinity and harboring a rare truncating pathogenic variant in exon 7 of the *SERPINF1* gene in a homozygous state.

Case Report

The patient was a 4-year-old female, born in a consanguineous family, with a history of delayed gross motor milestones and recurrent fractures after trivial trauma since the age of 8 months. The first fracture was a right femur subtrochanteric fracture at 8 months of age followed by a left femur subtrochanteric fracture at 12 months of age. The child was delivered vaginally at term with a birth weight of 3 kg. The patient had 48 h of NICU stay for neonatal

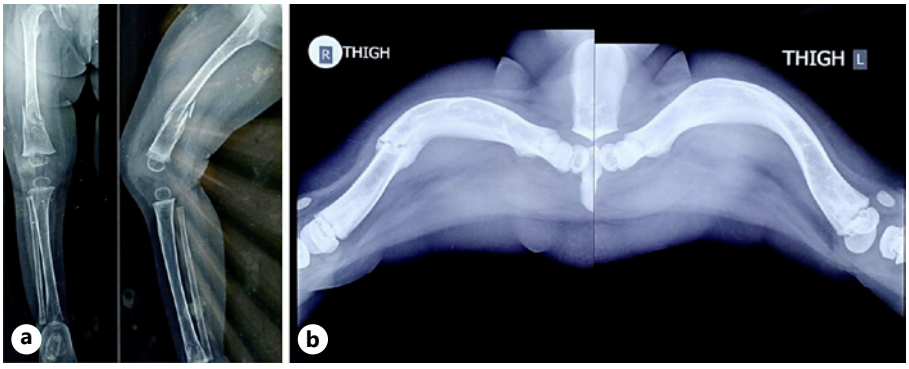

Fig. 1. X-ray of patient's femur (**a**) and thighs (**b**).

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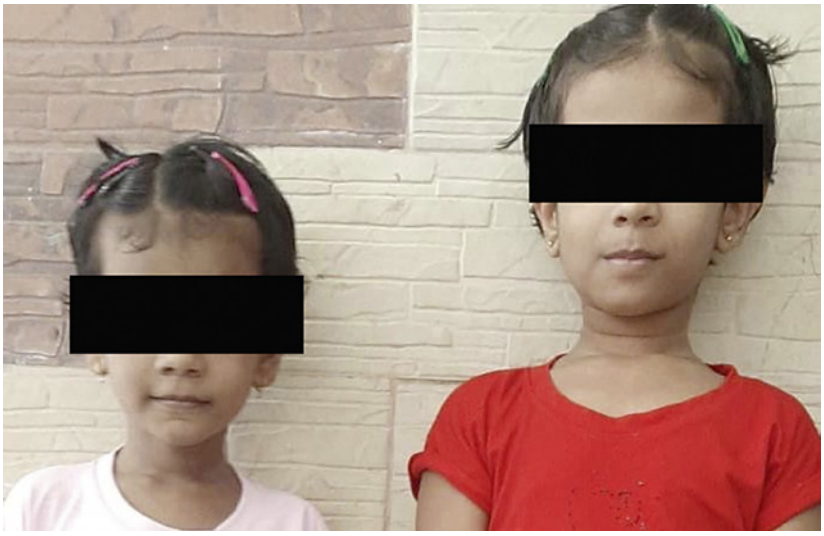

Fig. 2. Photograph showing triangular facies in the proband (right) and younger sibling (left).

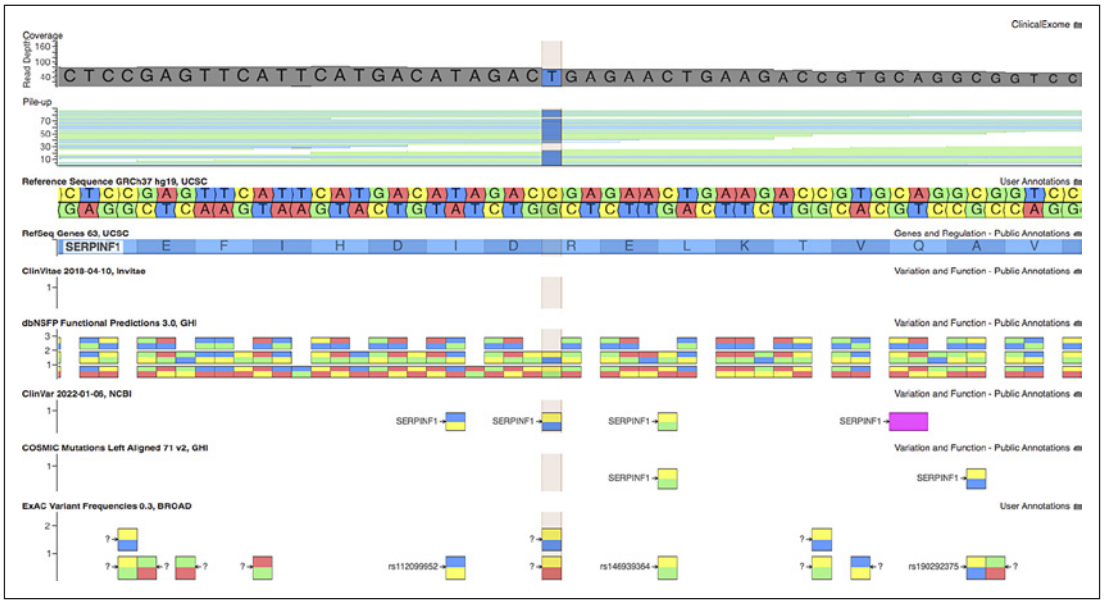

Fig. 3. NGS screenshot of *SERPINF1* gene sequencing.

hyperbilirubinemia. A skeletal survey of the patient was suggestive of bone fractures with callus formation, bilateral thigh deformities, and generalized osteopenia (Fig. 1). A bone scan showed abnormal moderate tracer uptake at the left humerus, left ribs, and bilateral femora. The patient underwent evaluation for recurrent fractures at 1.5 years of age. At the age of 1.5 years, the patient's weight was 6.4 kg (<3rd centile), height was 74 cm (less than 3rd centile for age and gender), and head circumference was 44 cm (5th centile). At 2.5 years of age, the patient's height was 77 cm and weight was 8.7 kg (both <3rd centile). These data indicated that the patient was underweight and of short stature, both of which are known to exist in OI [9, 10]. In addition, the patient had triangular facies (Fig. 2, right). These observations led to

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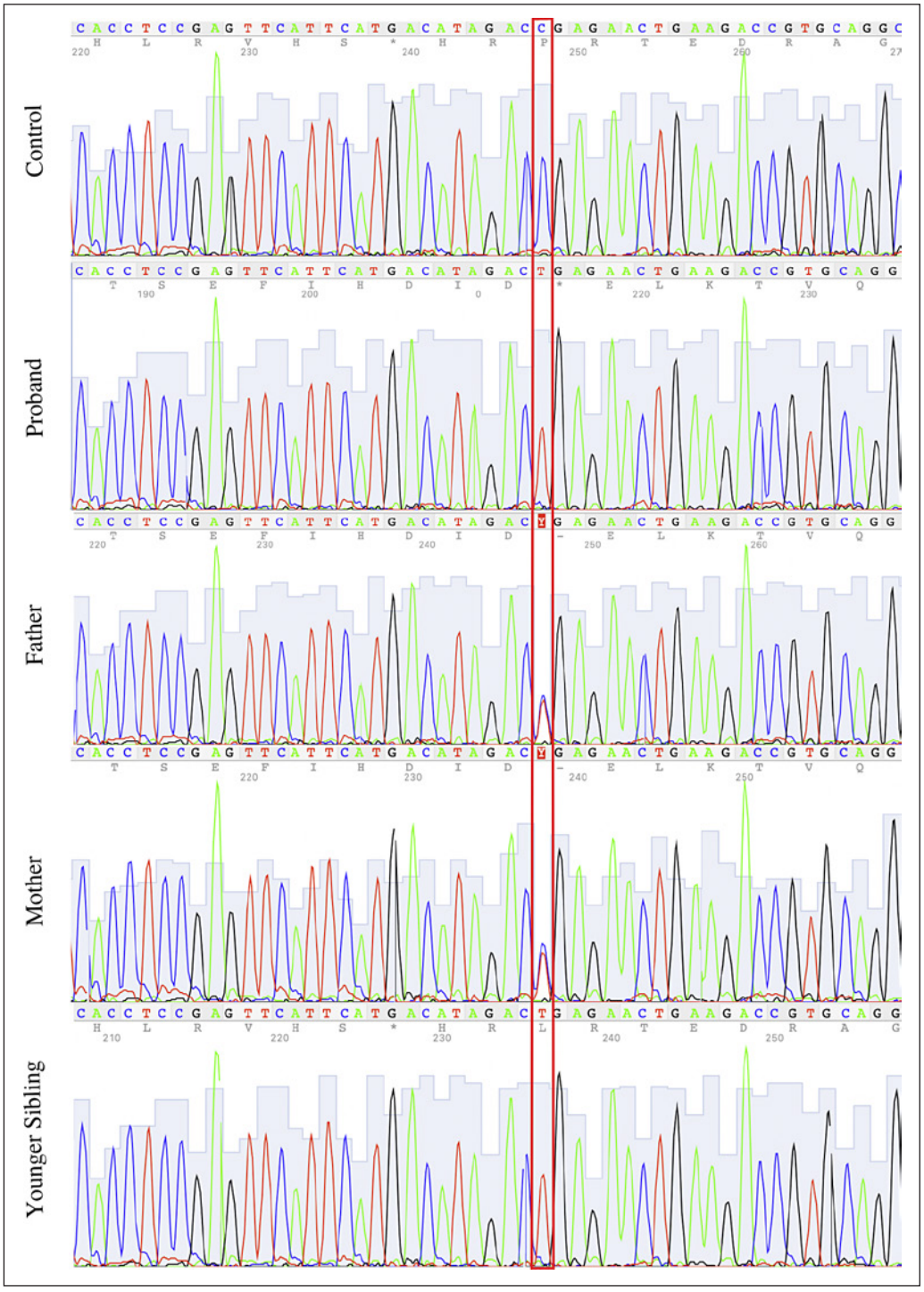

Fig. 4. Targeted capillary sequencing of the family's *SERPINF1* gene exon 7.

a clinical diagnosis of OI. Additionally, a skeletal survey of a 10-month-old younger sibling was indicative of osteopenia; however, no evidence of fractures or long bone deformities was found. The sibling had triangular facies (Fig. 2, left) and blue sclera, which are indicative of OI. Written informed consent was obtained from the patient's parents for publication of this case report and all accompanying images.

To obtain an etiological genetic diagnosis, an array of genes (*COL1A1, COL1A2, CRTAP, LEPRE1/P3H1, BMP1, FKBP10/BRKS1, SERPINF1, IFITM5, SERPINH1, ALPL, DTDST/SLC26A2, TRIP11, LRP5/OPPG*, and *PLOD2/BRKS2*) was analyzed by the TruSight One next-generation sequencing (NGS) panel. In short, DNA from peripheral blood of the patient was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) and quantified using the broad-range Qubit fluorometric assay (Thermo Fischer Scientific; catalogue #Q32853). The DNA was diluted as required and processed in the TruSight One NGS Panel (Illumina; Cat. # 15046895) on an Illumina sequencing-by-synthesis chemistry machine in paired-read mode. Bioinformatics analysis of the coding regions and exon-intron boundaries of the abovementioned genes, all of which are associated with different types of OI and a few other conditions with generalized osteopenia, was performed. The FASTQ sequence files generated by the Illumina MiSeq instrument were uploaded to the Illumina BaseSpace cloud analysis portal (Illumina, CA, USA). The sequences were aligned to the reference GRCH37/hg19 genome using the Burrows-Wheeler Aligner (BWA), and the resulting BAM files were analyzed for variants using the GATK Variant Caller (BaseSpace BWA Enrichment Workflow v2.1.1. with BWA 0.7.7-isis-1.0.0 and GATK v1.6-23-gf0210b3). The BAM and VCF files were visualized using GenomeBrowse v2.1.1 (Golden Helix, MT, USA). Bioinformatics analysis revealed the presence of a variant, NM_002615.5:c.907C>T (NP_002606.3:p.Arg303Ter), in exon 7 of the *SERPINF1* gene (ClinVar submission ID: SUB10491966) in a homozygous state (Fig. 3). The variant is predicted to result in the termination of the polypeptide chain at position 303. The variant was reported only once in the ExAC database (dbSNP: rs763291398), which is representative of the normal population, in a heterozygous state. The variant has been previously reported in association with OI by Li et al. [11] in 2019 and is also submitted in the LOVD database as pathogenic [12]. To confirm our NGS results, we designed custom primers such that we could perform targeted capillary sequencing of the region of interest. Targeted capillary sequencing confirmed the presence of this homozygous variant (Fig. 4; position highlighted by the red box) in the patient. Additionally, targeted capillary sequencing showed that both parents were heterozygous carriers for the variant (Fig. 4; position highlighted by the red box), confirming the allele segregation. Moreover, the same variant was detected in a homozygous state in the patient's younger sibling at the age of 10 months. The sibling was started on monthly bisphosphonate therapy; the sibling did not suffer any fractures in over 2 years after initiating the bisphosphonate therapy. Based on the genetic diagnosis, the family could be provided with genetic counseling, timely therapeutic intervention, and appropriate management to avoid fractures in the younger sibling.

Conclusion

We have reported a rare pathogenic variant in the *SERPINF1* gene in a case of early-onset severe OI with a history of consanguinity, confirming the diagnosis of type VI OI with autosomal recessive inheritance. OI type VI was first proposed by Glorieux et al. [4] in 2002 when they observed that a group of 8 patients shared a unique set of clinical features, including reduced bone density and frequent fractures. Initially classified as OI type IV, the authors felt that the clinical characteristics were unique enough to warrant a separate group. Serine protease inhibitors (SERPINs) play an important cellular role as they control the activity of serine proteases. There are over 37 reported human SERPINs divided into 9 subfamilies. Depending on function, they are broadly divided into inhibitory and non-inhibitory SERPINs; SERPINF1 belongs to the non-inhibitory class of SERPIN proteins [13]. The *SERPINF1* gene is 15,506 nucleotides long and has 8 exons. The functional protein is 418 amino acids long. The association of biallelic P/LP variants in the *SERPINF1* gene with OI type VI was first reported

by Homan et al. [14] in 2011. In recent years, multiple studies have ascertained multiple P/LP variants in the *SERPINF1* gene in patients with OI type VI. Zhang and colleagues reported a homozygous missense V356E (c.1067T>A) substitution and a homozygous p.Ala96_Gly215del (c.283 + 473_643 + 104del) mutation in two Chinese families [15]. Jin et al. [16] recently reported an intronic cryptic splice site, ENST00000254722.4:c.439 + 34C>T, leading to OI type VI. Ward and colleagues also reported another intronic variant (c.787-10C>G) that led to OI type VI in two children from Canada [17]. As of August 2022, 219 variants in the *SERPINF1* gene have been submitted to the ClinVar database [18]. Of these, 59 have been reported in association with OI type VI. As of August 2022, thirteen variants associated with OI have been reported in exon 7. Of these, only two are confirmed to be benign variants, with the rest being pathogenic or of uncertain significance [19]. Of the three pathogenic variants reported, one results in a frameshift mutation, whereas the other two result in nonsense mutations. Both the benign mutations result in synonymous variants. Of the eight variants of unknown significance, six result in missense variants, one results in a synonymous variant, and one in an in-frame deletion. Stephens et al. [20] have reported the *SERPINF1* p.(Phe277del) mutation in a homozygous state in an Indian patient with OI. This is a pathogenic mutation present in *SERPINF1* exon 7. Notably, the patient's fracture rate improved after starting bisphosphonate treatment, which is in agreement with observations regarding the patient's sibling in our case report.

One important feature of our report is that the NGS panel data of the proband were augmented with confirmatory targeted sequencing of the entire family. This helped us ascertain the autosomal inheritance pattern of the mutation. A potential limitation of our report is the absence of histological and biochemical data.

This report expands the clinical symptoms of OI type VI caused by a truncating mutation in *SERPINF1*. The mutation was harbored in a homozygous state in the proband and heterozygous state in the proband's parents. Additionally, this case report highlights the importance of clinical correlation with laboratory findings and the importance of a large NGS-based panel for simultaneous screening and diagnosis in cases with a common clinical presentation or phenotype. This case report has been written in accordance with the CARE checklist for case reports, which has been included in the online supplementary (for all online suppl. material, see www.karger.com/doi/10.1159/000527988).

Statement of Ethics

Written informed consent in compliance with the Helsinki Declaration was obtained from the patient's parents for publication of this case report and all accompanying images. Ethical approval is not required for this study in accordance with local or national guidelines as the patient identity is not revealed.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding Sources

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Author Contributions

Meenal Agarwal was the lead medical geneticist for the case, conceptualized the manuscript, and analyzed and interpreted the data. Siddharth Anand wrote the manuscript, assisted Meenal Agarwal in the molecular analysis, designed primers for targeted sequencing confirmatory analysis, and collected the data. Pratibha Pawal-Aute and Shashikant Gunale were the treating clinicians.

Data Availability Statement

The relevant data, i.e., the variant information has been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and can be accessed under the submission ID SUB10491966. Further enquiries can be directed to the corresponding author.

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