

Case study

Identification of osteogenesis imperfecta Type VI: A first case report from a Pakistani family

Abstract

Background: Osteogenesis imperfecta type VI (OI type VI) is a rare autosomal recessive disease of bone mineralization characterized by multiple fractures of bones after six months of age, without a history of other extra-skeletal complications. *SERPINF1* (serpin inhibitor clade F1) is the causative gene for this abnormality, having a chromosomal location as 17p13. Many cases have been reported from different populations of the world. No case has been reported from the population of Pakistan related to this deformity.

Case presentation: In the current study, we presented a case of Osteogenesis imperfecta type VI. The patient's clinical findings indicated her with short stature and progressive distortion of the skeleton, without the record of other complications like hearing problems, dental anomalies, and abnormal vision. She was 16 years old, could not walk due to deformation and weakness of lower limbs. There is a reported history of multiple fractures of long bones of the body at the time of radiological examination of the patient. The radiological findings showed the condition of **kyphoscoliotic** impairment in the **cervicodorsal** spine. Long bones show bowing and relatively decreased bone mineralization. Patients' sequencing data indicated a new homozygous frameshift mutation c.262_263insCCCTCTC (p. Ala91Profs*23) in *SERPINF1* responsible for splice site changes in PEDF protein.

Conclusion: This identified mutation was the first report from Pakistan, and an increase in the pathogenic variants in *SERPINF1* caused OI type VI.

Keywords: *SERPINF1*, consanguinity, novel frameshift, Osteogenesis imperfecta

Background

Osteogenesis imperfecta (OI) is a heterogeneous defect of bone fragility characterized by significant skeletal abnormalities like short stature and susceptibility to multiple fractures. Extra-skeletal complications like blue sclera, hypermobility of joints, renal dysfunction, and dentinogenesis imperfecta appeared in some cases depending upon the involvement of a specific gene (Cao *et al.*, 2019; Becker *et al.*, 2011; van Dijk *et al.*, 2011; Glorieux *et al.*, 2002). It is estimated that 6-7 individuals were affected in 10,000 live births with varying severity ratios from frequent fracture to intrauterine fracture and perinatal mortality (Minillo *et al.*, 2014). In OI type VI patients, multiple fractures were reported after six months, while other extra-skeletal complications were absent. Histological examination of bone depicted improper bone mineralization, accumulation of unmineralized osteoid, which gives a unique lamellar demonstration to bone (Zhan *et al.*, 2018; Tucker *et al.*, 2012; Homan *et al.*, 2011).

SERPINF1, a specific gene responsible for osteogenesis imperfecta type VI. It is localized on chromosome 17p13.3. PEDF (pigment epithelium-derived factor) is a 50-kDa secreted glycoprotein of the serpin superfamily. The various functionality of PEDF in cells is indicated as its involvement in multiple mechanisms as lipid metabolism, neuroprotection, anti-angiogenesis, and tumorigenesis. In bone, PEDF shows a crucial involvement in mineralization, osteoblast differentiation, and expression of osteocyte-related factors (Wang *et al.*, 2017; Trejo *et al.*, 2017; Rauch *et al.*, 2012; Homan *et al.*, 2011).

Up till now, 62 pathogenic variants in *SERPINF1* were detected, which affected about 58 individuals (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SERPINF1>) (https://oi.gene.le.ac.uk/home.php?select_db=SERPINF1). These mutations were reported from different populations of the world with diverse geography (Table 1). However, detailed investigations of OI type VI in the Pakistani population regarding phenotypes and gene mutations are still needed. In the present study, we analyzed phenotypic traits and pathogenicity of variants in Pakistani patients born due to an inter-family relationship between mother and father. Molecular analysis of coding regions of *SERPINF1* has indicated a non-reported homozygous in-frame insertion mutation c.262_263insCCCTCTC (p. Ala91Profs*23) in the enrolled patient.

CASE PRESENTATION:

A consanguineous Pakistani couple (IV-1, IV-2) (Figure 1) has a 16 years old female as 1st baby in their family. She was born as a result of a normal pregnancy by vaginal delivery. She was referred to University Hospital, The University of Lahore, for the initial clinical findings of OI by following criteria: frequent repetitive fractures under minor injuries with inevitable extra-skeletal distractions such as mild impairment of hearing and uncontrolled movements of joints but dentinogenetic imperfecta and blue sclera not observed in this patient. Her parameters at the time of birth, such as height, weight, and skull circumference, were recorded as usual. The family history indicted that one male in siblings was affected while others were healthy. The normal growth and development were recorded by parents, starting from her birth, still six months of age. After that, parents reported a history of arm and leg bone fractures with minor trauma. It was observed that patient had no free movements of the neck like normal individuals. But joints were not indicated any during the examination. Phenotypic appearance of facial deformity, teeth anomalies, and corneal defect were absent, but lower limbs showed a curvature shape (Figure 2: a and b). The patient was bearing normal mental health with excellent cooperative behavior. The patient's clinical examination history, radiological investigations, and pedigree analysis had confirmed the OI type VI rather than other types of OI. Bone deformities, hypermobility, and multiple fractures history differentiated it from different types. Radiological examination revealed a diffuse decreased density of the visualized bones, irregular articular surface.

X-ray films of the upper and lower extremities and thoracolumbar vertebrae were also examined. During an examination, marked **kyphoscoliotic** impairment in the **cervicodorsal** spine was observed. The extremities of long bones are frequently affected by fractures. The moderate-to-severe bone fragility resulted in short stature, decreased movements, and bone deformities (such as curvature of long bones, severe type of scoliosis, and kyphosis). Furthermore, the major recorded observations of skinny long bone and compressions in vertebrae and infinite osteoporosis were observed (Figure 3: a-d). Long bones showed bowing, thinning of cortices, metaphyseal flaring, and relatively decreased bone mineralization. The collected family samples were processed for sequencing of *SERPINF1* to identify the genetic mutation of this abnormality and for possible preventive measures for its management.

Sanger sequencing technique was used for the identification of a pathogenic variant in *SERPINF1* and co-segregation analysis in the family. *SERPINF1* genomic transcript (NM_002615.5) and the mutation nomenclature of the gene have been extracted from the genome database browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>). An updated software “AmplifX v1.5.4” was used for primers designing (<http://crn2m.univ-mrs.fr/pub/amplifx>), and PCR was carried out for the amplification of coding regions. ExoSap protocol (<https://www.thermofisher.com>) was performed for the Cleanup of PCR products. An ABI 3730 genetic analyzer with BigDye chemistry v3.1 was used for sequencing. The obtained chromatograms were altered with the reference sequence based on SeqMan Pro (DNASTAR, Inc., Madison, WI, UK) software. Analysis has confirmed the allelic frequency of the identified pathogenic variant by taking the help of the Genome aggregation database (gnomAD, <http://gnomAD-old.broadinstitute.org/>).

The coding sequences (eight exons) of *SERPINF1* were PCR amplified for the patient (V-1) DNA samples. The sequence analysis results indicated a novel homozygous 7bp in-frame insertion c.262_263insCCCTCTC (p. Ala91Profs*23) in the *SERPINF1* gene (Figure 4). The sequencing of identified pathogenic variants was carried out for both parents (IV-1, IV-2).

Discussion and Conclusion

In this study, we reported a consanguineous Pakistani family with one affected member (V-1) (Figure 1) with a history of frequent fractures under mild trauma and certain other skeletal deformities on later stages of life presented as a patient of OI type VI. The clinical laboratory findings coincided with the apparent phenotypic appearance of a female patient. The thorough examination of parents (IV-1, IV-2) and two unaffected brothers (V-3 and V-4) revealed the absence of major symptoms of Osteogenesis Imperfecta (OI). Sanger sequencing revealed a novel homozygous frameshift mutation c.262_263insCCCTCTC (p. Ala91Profs*23) in the *SERPINF1* gene, which may lead to splice site changes in PEDF. Previously, few mutations in the *SERPINF1* gene have been in OI type VI in different world populations like Chinese, Brazilian, Arab, Korean populations, etc. (Table 1).

Patients (OI) are categorized into different types based on the disease's severity and specific inheritance pattern. 90% of cases of OI reported as autosomal dominant are related to aberrations in two genes translated into type I collagen, such as COL1A1 or COL1A2. Autosomal

recessively inherited OI types comprised 10 %. Type VI (MIM #610968) is a less frequent form resulting from pathogenic variations of the *SERPINF1* gene (Wang *et al.*, 2017; Minillo *et al.*, 2014; Beaker *et al.*, 2011). OI type VI was recognized as a different type a decade ago. It has a 4% estimated occurrence ratio in the human population (Rauch and Glorieux, 2004; Homan *et al.*, 2011; Rauch *et al.*, 2012).

As described in previous studies, clinical examination of the current case showed no corneal defect and teeth anomalies in the affected individual (Homan *et al.*, 2011; Rohrbach *et al.*, 2012; Minillo, 2014). Previous *in vitro* studies demonstrated that the density of bone is defected by PEDF inhibition of osteoclasts (Akiyama *et al.*, 2010). The phenotypically appearance of frequent bone fractures after six and absence of extra-skeletal defects differentiate it from others types of OI (Homan *et al.*, 2011; Rohrbach *et al.*, 2012).

The primary causative gene (*SERPINF1*) for this specific type of osteogenesis has a chromosomal location as17p13.3, which translates into a unique (PEDF) glycoprotein containing 418 amino acids (Homan *et al.*, 2011; Becker *et al.*, 2011; Rauch *et al.*, 2012; Al-Jalladet *al.*, 2015). It has a diverse functionality with expression in vast tissues such as the adult brain, spinal cord, plasma, lung, eye, heart, and bone (Tucker *et al.*, 2012).

In the knockout mouse model (*Serpinf1*^{-/-}) presence of vast deposits of unmineralized bone, the matrix built a resemblance with phenotypes of OI type VI (Wang *et al.*, 2017).

Some frame insertion and deletion mutations have also been published along with previous ones responsible for premature termination codons (Al-Jalladet *al.*, 2015).

Most of the reported pathogenic variants in *SERPINF1* are frameshift and nonsense mutations that cause defects in the normal functionality of PEDF by altering the protein network (Wang, 2017). Previously reported mutations were collected https://oi.gene.le.ac.uk/variants.php?action=search_unique&select_db=SERPINF1.

The current study described a case of osteogenesis imperfecta OI. The molecular clinical study of a Pakistani patient born due to an inter-family union confirmed pathogenic mutation causing type VI of OI bone disorder. The current study's findings are novel and considered an addition to the mutational spectrum of the *SERPINF1* gene.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by any funding agency rather it was funded by personal efforts of the authors.

UNDER PEER REVIEW

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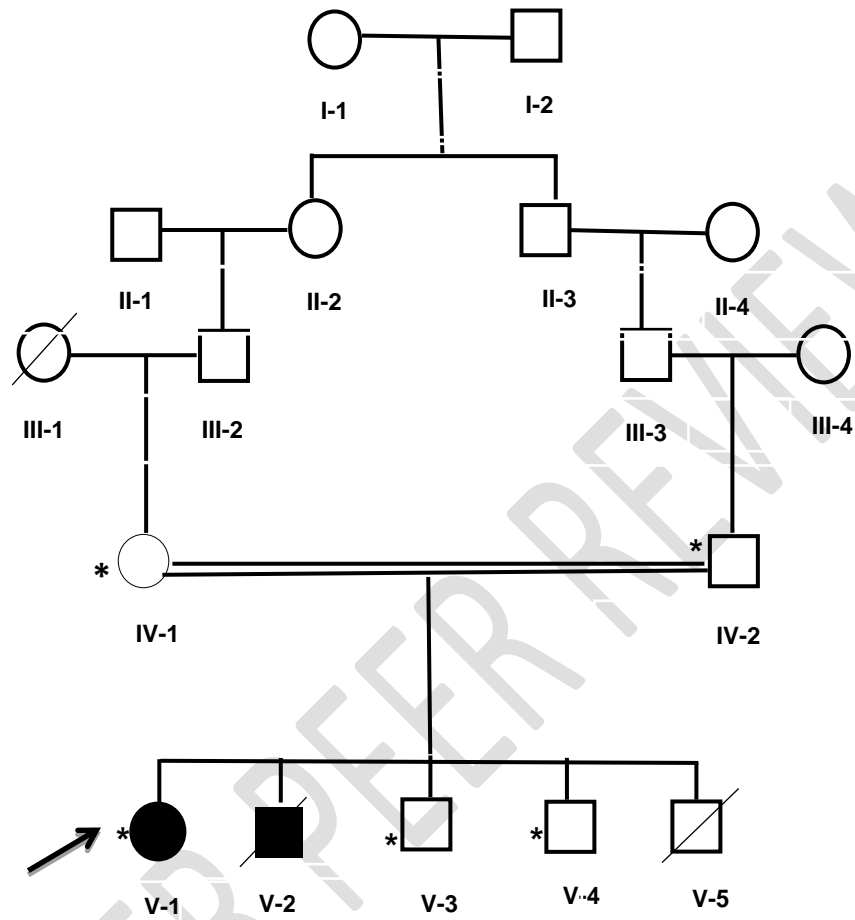


Figure 1: Pedigree of a Pakistani family segregating an autosomal recessive OI type VI. Arrow indicates the index patient (V-1). The samples which were available for the genetic analysis are marked with asterisks (*).



Figure 2: (a, b) Normal facial features of the patients, who was a 16 years old girl with sever deformity in lower limbs presenting her unable to sit or walk.

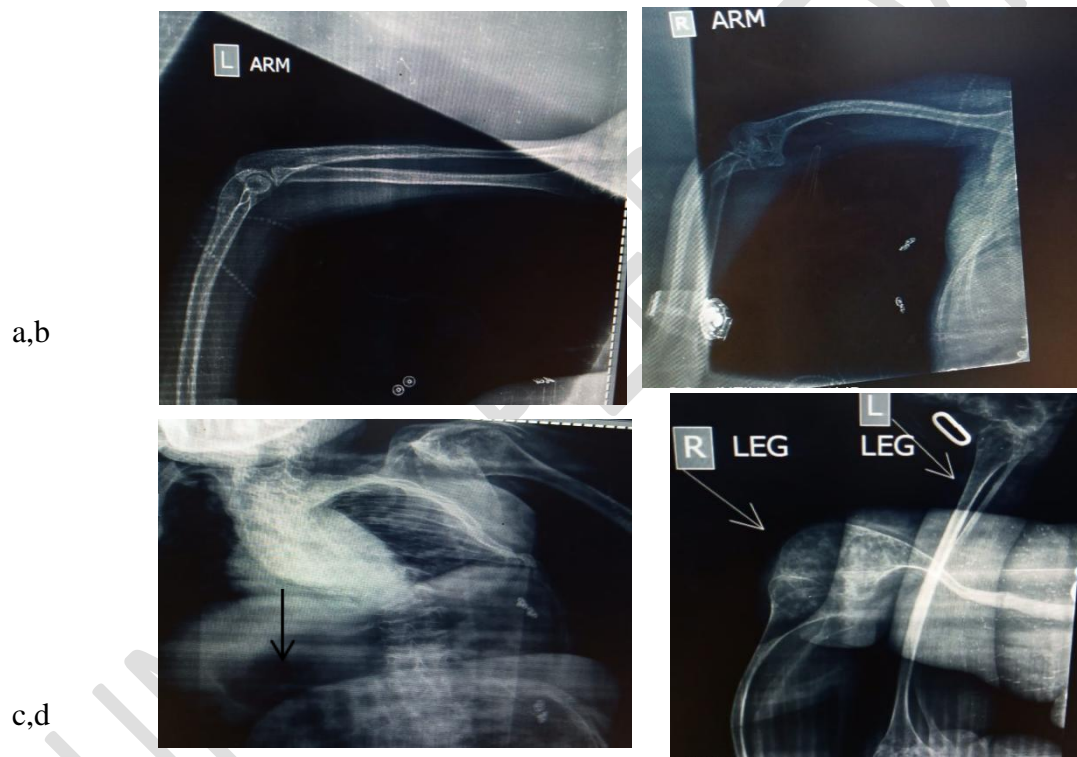


Figure: 3 Radiological examination of upper and lower limbs and vertebrae indicating lower limbs were present in a curve shape structure, decrease bone density at edges and bending of vertebrae

Table 1: A list of significant variants in hotspot region of *SERPINE1* gene responsible for Osteogenesis Imperfecta (OI)

C hr	Gene	hg19 Position	Ref - allele	Alt_allele	Gene Bank	cDNA change	Amino acid change	Type of variant	Dis ease	dbSNP_rsID	MAF gnomAD Genome	MAF gnomAD Exome	Reference
17	SE RPI NF 1	1673320	A	ACGGCCCTCT	NM_002615	c.259_260insC GGCCCTCT	p.Ala91_Ser93dup	Infra me Insertion	NA	rs373146540	0.00006369	0.00005570	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673320	A	ACGGCCCTCTCGGCCCTCT	NM_002615	c.259_260insC GGCCCTCTC GGCCCTCT	p.Ala88_Ser93dup	Infra me Insertion	NA	rs373146540	NA	0.000003979	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673320	A	T	NM_002615	c.259A>T	p.Thr87Ser	Misse nse	NA	rs373146540	NA	0.00002387	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673320	A	CGGCCCTCTCT	NM_002615	c.259_260insC GGCCCTCT	p.Ala91_Ser93del	Infra me Deletion	NA	rs758551389	NA	0.00001592	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673321	C	T	NM_002615	c.260C>T	p.Thr87Met	Misse nse	NA	rs768284337	NA	0.00001991	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673321	C	GGCCC	NM_002615	c.261_265dupG GCCC	p.Leu89Argfs*26	Frame shift Insertion	OI III	NA	NA	NA	Li et al., 2019
17	SE RPI NF 1	1673323	G	GCCCTCTCG	NM_002615	c.271_279dupG CCCTCTCG	p.Ala91_Ser93dup	Infra me Insertion	OI VI	NA	NA	NA	Tucker et al., 2012
17	SE RPI NF 1	1673324	G	CCCTCTC	NM_002615	c.262_263insC CCTCTC	p.Ala91Profs*23	Frame shift Insertion	OI VI	NA	NA	NA	Present study
17	SE RPI NF 1	1673330	C	G	NM_002615	c.269C>G	p.Ser90Trp	Misse nse	NA	rs144853088	NA	0.000003982	https://gnomad.broadinstitute.org/gene/ENSG00000132386

17	SE RPI NF 1	1673 330	C	T	NM_0 02615	c.269C>T	p.Ser9 0Leu	Misse nse	N A	rs14 4853 088	NA	0.0000159 3	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SE RPI NF 1	1673 333	C	G	NM_0 02615	c.272C>G	p.Ala9 1Gly	Misse nse	N A	rs76 5086 186	NA	0.0000039 82	https://gnomad.broadinstitute.org/gene/ENSG00000132386

Parents
(carrier)

Parents
(carrier)

affected

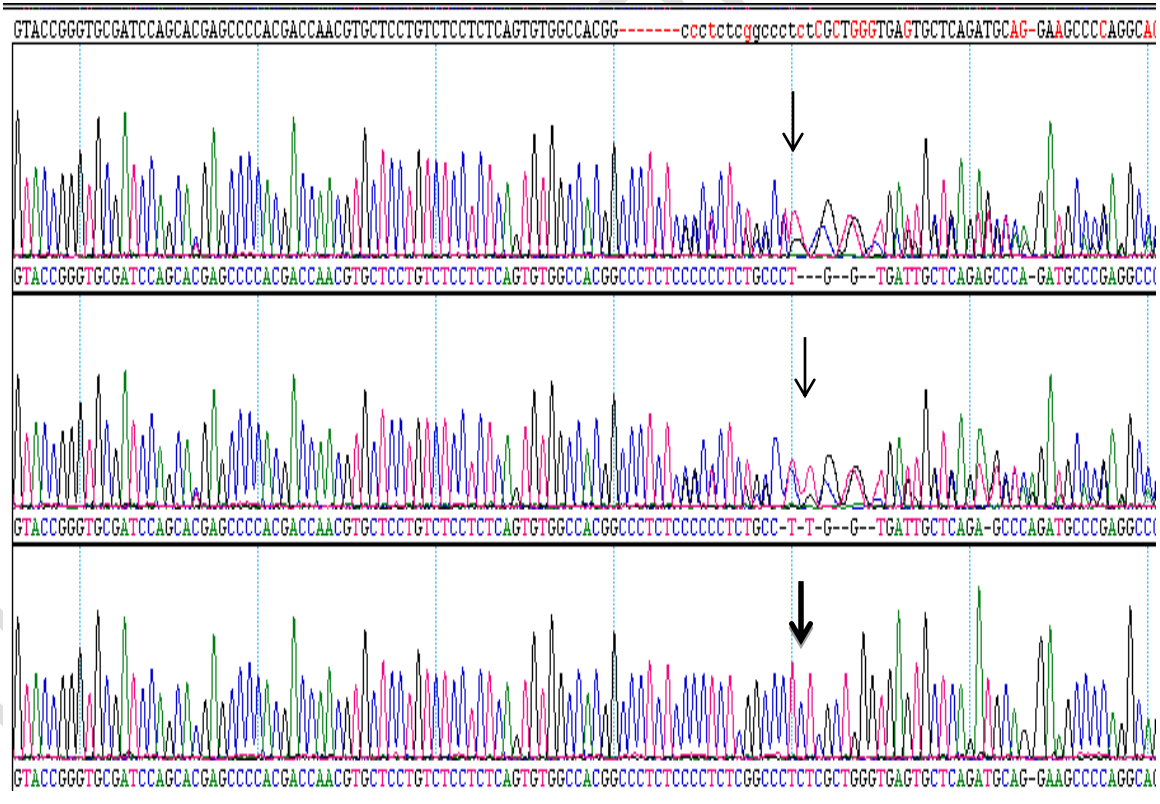


Figure: 4 Sequence analysis of *SERPINE F1* causing in frame insertion c.262_263insCCCTCTC (p.Ala91Profs*23)