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, 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 Pakistani population 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. History of multiple fractures of long bones of the body was reported at the time of radiological examination of the patient. The radiological findings showed the condition of kyphosocolitic impairment in the cervicodorsoliotic 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 major 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 un-mineralized osteoid, which gives a unique lamellar demonstration to bone (Zhang 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 performed an analysis of 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 normal. The family history was indicted as one other 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 had a history of arm and leg bone fractures at minor trauma. There 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 b). The patient was bearing normal mental health with excellent cooperative behavior. Clinical examination history, radiological investigations, and pedigree analysis of the patient were led towards the confirmation of OI type VI this affected member 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. Marked kyphosocolitic impairment in the cervicodorsoliotic spine was examined. 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 (Figure 3: a-d). Long bones show 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 abnormity and for possible preventive measures for its management.

Identification of a pathogenic variant in *SERPINF1* and co-segregation analysis in the family done by Sanger sequencing technique. *SERPINF1* genomic transcript (NM_002615.5) and the mutation nomenclature of the gene have been extracted from genome database browser (http://genome.ucsc.edu/cgibin/hgGateway). An updated software "AmplifX v1.5.4" was used for primers designing (http://crn2m.univ-mrs.fr/pub/amplifx), and PCR carried out amplification of coding regions. Cleanup of PCR products by ExoSap protocol (https://www.thermofisher.com) was performed. 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. Allelic frequency of the identified pathogenic variant was confirmed 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 sample. 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. A. The thorough examination of parents (IV-1, IV-2) and two unaffected brother (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 populations of the world like chines, Brazilian, Arab, Korean population, etc. (Table 1)

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

recessively inherited OI types comprised of 10 %. Type VI (MIM #610968) is a less frequent form resulting from pathogenic variations of *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 study demonstrated that the density of bone is defected by PEDF inhibition of osteoclasts (Akiyama et al., 2010). 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 main causative gene (*SERPINF1*) for this specific type of osteogenesis has a chromosomal location as17p13.3, which translated into a unique (PEDF) glycoprotein containing 418 amino acids. (Homan et al 2011; Becker et al., 2011; Rauch et al., 2012; Al-Jallad et al., 2015). It has a diverse functionality with expression in vast tissues as the adult brain, spinal cord, plasma, lung, eye, heart, and bone. (Tucker et al., 2012).

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

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

Most of the reported pathogenic variants in *SERPINF1* are frameshift and nonsense mutations that causes 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 as a result of an inter-family union and the confirmation of 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 use 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 the producing company rather it was funded by personal efforts of the authors.

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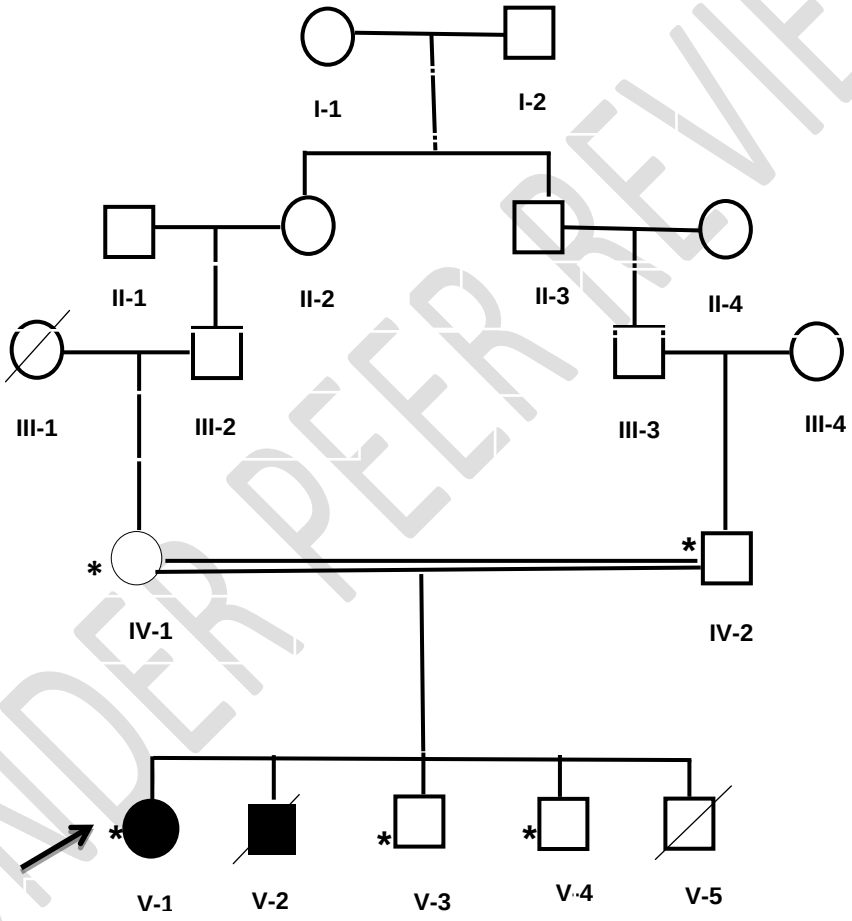
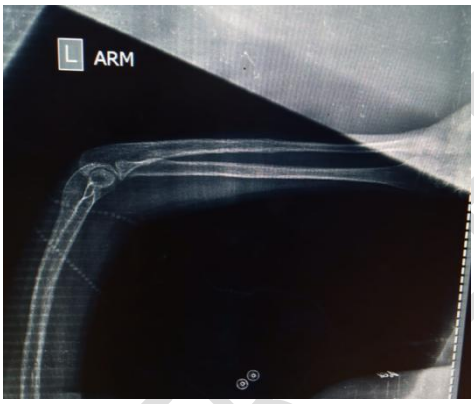


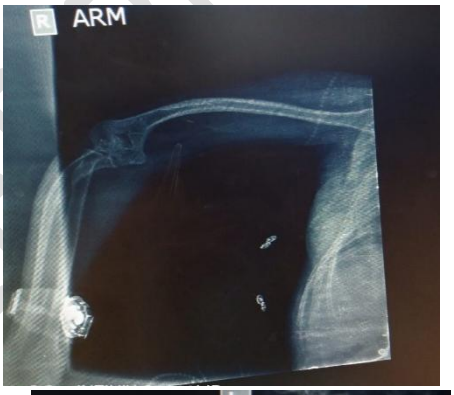
Figure 1: Pedigree of a Pakistani family segregating an autosomal recessive OI type VI. Arrow indicates the index patient (V-1). The samples which were available for the genetic analysis are marked with asterisks (*).

a   b

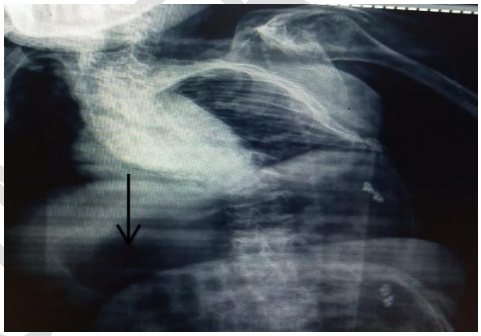
Figure 2: (a, b) Normal facial features of the patients, who was a 16 years old girl with sever deformity in lower limbs presenting her unable to sit or walk.

a,b





c,d



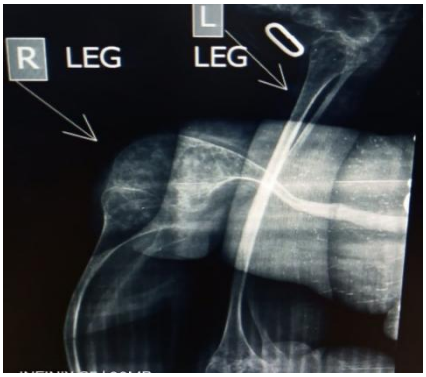


Figure: 3 Radiological examination of upper and lower limbs and vertebrate indicating lower limbs were present in a curve shape structure, decrease bone density at edges and bending of vertebrae

Table 1: A list of significant variants in hotspot region of *SERPINEF1* gene responsible for Osteogenesis Imperfecta (OI)

Chr	Gene	hg19 Position	Ref_allele	Alt_allele	Gene Bank	cDNA change	Amino acid change	Type of variant	Disease	dbSNP_rsID	MAF gnomAD Genome	MAF gnomAD Exome	Reference
17	SERPINF1	167320	A	ACGGCCCTCT	NM_002615	c.259_260insCGGCCCTCT	p.Ala91_Ser93dup	Inframe Insertion	NA	rs373146540	0.00006369	0.00005570	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167320	A	ACGGCCCTCTCGGCCCTCT	NM_002615	c.259_260insCGGCCCTCTCGGCCCTCT	p.Ala88_Ser93dup	Inframe Insertion	NA	rs373146540	NA	0.00000397	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167320	A	T	NM_002615	c.259A>T	p.Thr87Ser	Missense	NA	rs373146540	NA	0.00002387	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167320	ACGGCCCTCT	A	NM_002615	c.259_260insCGGCCCTCT	p.Ala91_Ser93del	Inframe Deletion	NA	rs758551389	NA	0.00001592	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167321	C	T	NM_002615	c.260C>T	p.Thr87Met	Missense	NA	rs768284337	NA	0.00001991	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167321	C	GGCCC	NM_002615	c.261_265dupGGCCC	p.Leu89Argfs*26	Frameshift Insertion	OI III	NA	NA	NA	Li et al., 2019
17	SERPINF1	167323	G	GCCCTCTCG	NM_002615	c.271_279dupGCCCTCTCG	p.Ala91_Ser93dup	Inframe Insertion	OI VI	NA	NA	NA	Tucker et al., 2012
17	**SERPINF1**	**167324**	**G**	**CCCTCTC**	**NM_002615**	**c.262_263insCCCTCTC**	**p.Ala91Profs*23**	**Frameshift Insertion**	OI VI	**NA**	**NA**	**NA**	**Present study**
17	SERPINF	167330	C	G	NM_002615	c.269C>G	p.Ser90Trp	Missense	NA	rs144853	NA	0.00000398	https://gnomad.broadinstitute.org/gene/

	1									088			ENSG00000132386
17	SERPINF1	167330	C	T	NM_002615	c.269C>T	p.Ser90Leu	Missense	NA	rs144853088	NA	0.00001593	https://gnomad.broadinstitute.org/gene/ENSG00000132386
17	SERPINF1	167333	C	G	NM_002615	c.272C>G	p.Ala91Gly	Missense	NA	rs765086186	NA	0.000003982	https://gnomad.broadinstitute.org/gene/ENSG00000132386

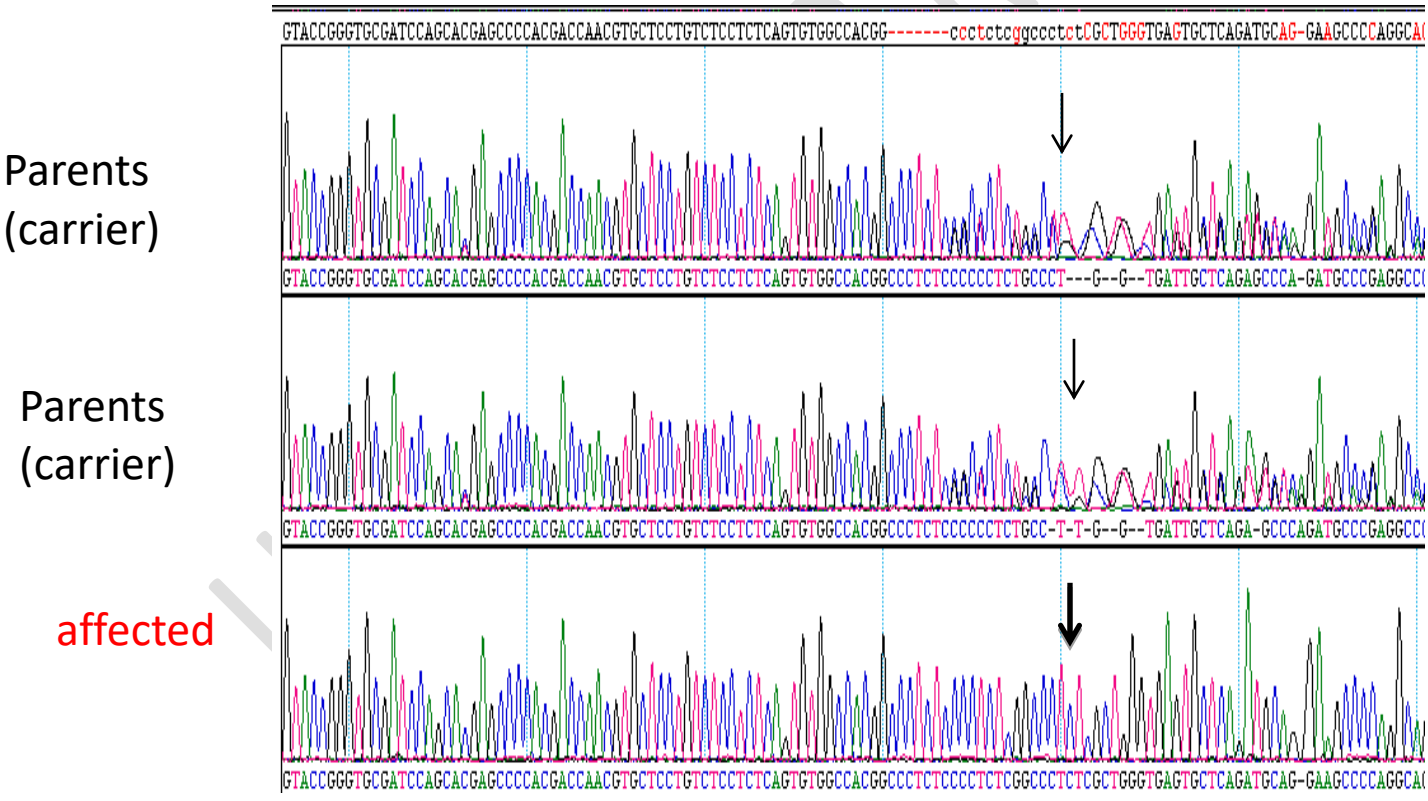


Figure: 4 Sequence analysis of *SERPINE F1* causing in frame insertion c.262_263insCCCTCTC (p.Ala91Profs*23)