

PHENOTYPIC VARIABILITY IN INDIVIDUALS WITH TYPE V OSTEOGENESIS IMPERFECTA WITH IDENTICAL IFITM5 MUTATIONS

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ABSTRACT

Background: Osteogenesis imperfecta (OI) type V is a dominantly inherited skeletal dysplasia characterized by fractures and progressive deformity of long bones. In addition, patients often present with radial head dislocation, hyperplastic callus, and calcification of the forearm interosseous membrane. Recently, a specific mutation in the *IFITM5* gene was found to be responsible for OI type V. This mutation, a C to T transition 14 nucleotides upstream from the endogenous start codon, creates a new start methionine that appears to be preferentially used by the translational machinery. However, the mechanism by which the lengthened protein results in a dominant type of OI is unknown.

Methods and Results: We report 7 ethnically diverse (African-American, Caucasian, Hispanic, and African) individuals with OI type V from 2 families and 2 sporadic cases. Exome sequencing failed to identify a causative mutation. Using Sanger sequencing, we found that all affected individuals in our cohort possess the c.-14 *IFITM5* variant, further supporting the notion that OI type V is caused by a single, discrete mutation. Our patient cohort demonstrated inter- and intrafamilial phenotypic variability, including a father with classic OI type V whose daughter had a phenotype similar to OI type I. This clinical variability suggests that modifier genes influence the OI type V phenotype. We also confirm that the mutation creates an aberrant IFITM5 protein containing an additional 5 amino acids at the N-terminus.

Conclusions: The variable clinical signs in these cases illustrate the significant variability of the OI type V phenotype caused by the c.-14 *IFITM5* mutation. The affected individuals are more ethnically diverse than previously reported.

BACKGROUND

Osteogenesis imperfecta (OI) is a clinically variable and genetically heterogeneous fragile bone disorder made up of at least 10 distinct subtypes and caused by mutations in 12 genes.¹⁻³ OI subtypes I to IV are distinguished by clinical criteria and account for >95% of all OI cases. These subtypes result from dominant mutations in the genes for type I collagen, COL1A1 and COL1A2. Recently, the molecular and functional basis of several recessive OI subtypes has also been described. The causative genes encode proteins involved in osteoblast differentiation or the folding and posttranslational modification of type I collagen proteins during assembly.

OI type V is a dominantly inherited variant of OI first described in 2000.⁴ Patients present with increased fractures of long bones and vertebral bodies. In addition, affected individuals often have hyperplastic callus and calcification of the interosseous membrane of the forearm and a radiodense metaphyseal line. Dentinogenesis imperfecta and blue sclera, which are present in other forms of dominantly inherited OI, are absent.⁵⁻⁹ Unlike the other dominant OIs, type V is not caused by mutations in the COL1A1 or COL1A2 genes. In August 2012, two groups reported that a mutation in the *IFITM5* gene is responsible for OI type V.^{10,11}

Remarkably, all affected individuals in 21 unrelated familial and sporadic cases carried exactly the same mutation: a C>T transition 14 nucleotides upstream from the start of the IFITM5 coding region. The mutation encodes a new start methionine 5 amino acids upstream from the endogenous start site. Four subsequent publications described an additional 65 patients with OI type V who also carried the same IFITM5 C>T variant [4-9].¹⁰⁻¹⁵ Interestingly, the spectrum for the IFITM5 variant may be broader than the OI type V phenotype, because the same variant was reported in an individual with type III/IV OI.¹⁶

We report that our cohort of ethnically diverse individuals with OI type V carry identical c.-14 IFITM5 mutations. However, the presence of marked inter- and intrafamily variability suggests that additional genes modify the severity of the OI type V phenotype.

MATERIALS AND METHODS

Patients

Written informed consent was obtained from all study participants.

IFITM5 cDNA cloning

Human IFITM5 cDNA was polymerase chain reaction–amplified from oligo-dT primed SAOS-2 cDNA and cloned into the pCR2.1-TOPO TA cloning vector to create the wild-type (WT) cDNA. The mutant (MUT) cDNA was generated using a specific 5' primer that included the modified sequence. Both cDNAs were verified by Sanger sequencing and then subcloned into pcDNA6/V5-His (Invitrogen) for the expression of C-terminally V5-His tagged IFITM5 in mammalian cells.

For cell transfection studies, HEK293 cells were grown to 90% confluence in Dulbecco's modified Eagles medium supplemented with 10% fetal calf serum and transiently transfected overnight using Lipofectamine 2000 (Invitrogen). Cells were transfected with empty vector (pcDNA6), WT IFITM5, or MUT IFITM5 constructs. Cells were lysed in NP40 lysis buffer 48 hours later, and proteins were separated by SDS-PAGE under reducing conditions using a 12% resolving gel followed by Western blot using an anti-V5 antibody (AbD Serotec). Proteins were imaged using the LI-COR Odyssey infrared system.

Sequencing

Prior to the first report of the gene for OI type V,^{10,11} exome sequencing was conducted on 4 individuals from family 1 (**Figure 1**). Exome sequences were captured using the Agilent V1.0.1 SureSelect exon capture library and sequenced on an Illumina HiSeq 2000 machine. Following the identification of IFITM5 as the causative gene for type V OI, all patients were sequenced for the reported variant by Sanger sequencing.

RESULTS AND DISCUSSION

Our OI type V patient cohort consisted of 7 affected individuals in 2 unrelated families and 2 sporadic cases (**Figure 1**). The ethnic backgrounds of the affected individuals in this study are African-American (family 1), white (family 2), Hispanic, and African. We initially screened 4 individuals from the African-American family (individuals I-I, II-I, II-III, II-IV) by whole exome sequencing. While we identified several promising variants in the family, none segregated with the affected

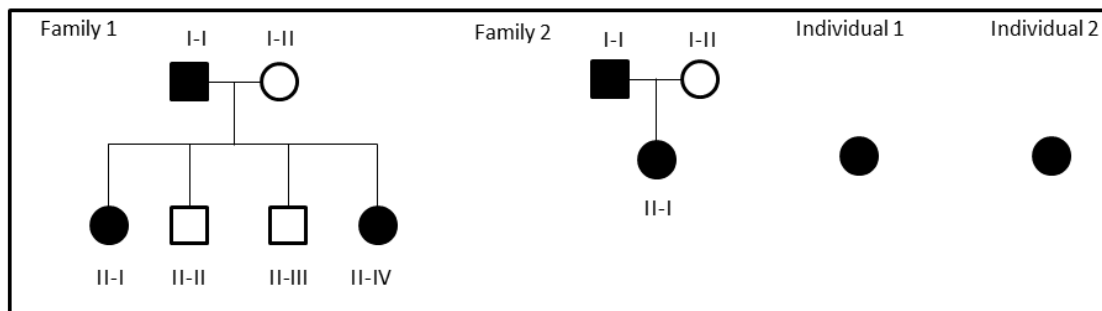


Figure 1. Pedigrees of osteogenesis imperfecta type V families and individuals in this study. Sanger sequencing confirmed that all affected individuals (black circles [female] and squares [male]) contain the c.-14C>T *IFITM5* variant and all unaffected individuals (white circles and squares) are wild-type for the variant Roman numerals used in the Figure, Table and text identify individuals within each family.

individuals in 4 additional unrelated persons. Following our exome analyses, a variant in the 5' untranslated region of the IFITM5 gene was reported to cause OI type V.^{10,11,14,15} Manual Sanger sequencing of the IFITM5 gene in our cohort revealed that all affected individuals had the same heterozygous c.-14C>T change reported and no unaffected family members had the variant. The failure to identify the IFITM5 mutation by exome capture and sequencing was explained by the poor capture of exon 1 of IFITM5. This highlights a weakness of exome sequencing strategies, in which exonic fragments are captured with variable efficiency. Interestingly, the studies of Shapiro et al¹⁴ and Rauch et al¹⁵ also failed to identify the pathogenic IFITM5 variant using an exome sequencing approach.

Echoing the observations of 2 previous reports,^{14,15} the patients exhibited some clinical variation despite the presence of an identical mutation (**Table**). Between the 2 families and the 2 sporadic cases, there are differences in levels of physical activity, scleral hue, gross motor function, and presence of calcified membrane interossea, hyperplastic callus, and scoliosis. Even within families there are several features that vary. Two sisters in family 1 have similar presentations overall but differ in the presence/absence of hypermobile joints and radial head dislocation. These differences in presentation between family members is consistent with the finding of Rauch et al, who reported interindividual variability in disease severity even within the same family.¹⁵

In family 2, the father presents with clearly defined OI type V with reduced joint mobility due to callus formation. His daughter has features more typically associated with "classic" type I OI, including mild dentin changes and height within the normal range. She had previously been excluded for COL1A1 and COL1A2 mutations. At initial presentation, she had no clinical or radiologic evidence of intramembranous calcification. However, she has developed limited supination and the characteristic calcification in adolescence. She lacks hyperplastic callus formation. The striking differences in presentation between the father (OI type V) and daughter (with features primarily associated with OI type I) in family 2 may suggest that the OI type V phenotype develops later during childhood. Alternatively, the presence/absence of modifying genes derived from the maternal line contributes to the variable clinical presentation. Interestingly, the IFITM5

variant was reported in a child lacking any features of OI type V.¹⁶ Instead, the patient presented with OI type III or severe OI type IV. It remains to be seen how broad the IFITM5 clinical spectrum is, and we suggest that all individuals with OI with unknown genetic etiology be screened for the IFITM5 variant.

Our report brings the total number of patients with OI type V with this particular mutation to 86, highlighting the complete lack of genetic heterogeneity in this disorder. However, the finding that a single mutation appears to account for all OI type V cases worldwide makes it relatively straightforward to screen suspected cases, allowing precise molecular diagnosis and potentially streamlining therapeutic options.

IFITM5 is a bone-specific protein whose function is poorly understood, and as such, the molecular and cellular consequences of the IFITM5 mutation remain unresolved. IFITM5 is clearly involved in bone mineralization. IFITM5 mRNA and protein are present at newly formed bone surfaces during development in mouse embryos, with the highest levels of IFITM5 in osteoblasts associated with the onset of matrix maturation and mineralization.¹⁷ Furthermore, overexpression of IFITM5 enhances mineralization and knockdown of IFITM5 gene expression by short-hairpin RNA reduced mineralization in osteoblast cell lines. However, IFITM5 is not essential for bone formation and mineralization in mice,¹⁸ as *Ifitm5* gene knockout mice were found to be relatively normal, with undermineralized cranial bones and shorter long bones being the main skeletal defects.

As shown in **Figure 2** and elsewhere,^{10,11} the c.-14C>T IFITM5 variant found in all patients with OI type V creates a new start methionine upstream from the endogenous start site, extending the N-terminus by 5 amino acids (Met-Ala-Leu-Glu-Pro), and decreases its mobility, as determined by SDS-polyacrylamide gel electrophoresis. However, a lack of a fundamental understanding of IFITM5 function makes it difficult to speculate how the altered protein causes the reported mineralization and bone repair abnormalities. Several basic questions remain unanswered. For example, the IFITM5 protein has 2 transmembrane domains with both the N- and C-termini predicted to extend into the extracellular space.¹⁷ However, alternative topologies have been suggested that place the N-terminus on the

Table. Clinical Features of the Type V Osteogenesis Imperfecta Cohort

Characteristic	Family 1: Patient II-I	Family 1: Patient II-IV	Family 2: Patient II-I	Family 2: Patient I-I	Individual 1	Individual 2
Age	10	4	9	47	21	24
Sex	female	female	female	male	female	female
Ethnicity	African-American	African-American	white	white	Hispanic	African
Height	<5th percentile	<5th percentile	nl	<5th percentile	<5th percentile	<5th percentile
No. of fractures	>10	>4	14	multiple	n/a	>10
Hyperplastic callus	yes	yes	no	yes	yes	yes
Disability other than forearm or elbow	no	n/a	no	yes	yes	yes
Physical activity	nl	possibly limited	n/a	limited	limited as child but more nl as adult	limited
Age at start of bisphosphonate treatment	no treatment	no treatment	9 mo	n/a	n/a	Forteo® (teriparatide) study/18 y
Confirmed prenatal fractures	no	no	no	n/a	none reported	none reported
Age at first non-traumatic fracture	4 mo	3 mo	6 mo	n/a	n/a	3 mo
Color of sclera	blue/grey	not available	nl	nl	n/a	nl
Dentinogenesis imperfecta	nl	nl	no, mild dentin changes	nl	nl	nl
Hearing impaired	no	no	no	no	no	no
Calcified membrane interossea	no	no	yes (in adolescence)	yes	yes	yes
Severe bowing of the extremities	bowing, due to fractures	bowing	no	bowing, due to fractures	bowing	bowing
Reduced joint mobility due to callus	yes	n/a	yes	yes	yes	yes
Retarded gross motor function	no	possible, not walking at 16 mo	no	yes	none reported	yes
DXA (most recent)	n/a	n/a	-0.7	n/a	2011 total body z score: -1.3	n/a
Scoliosis	no	no	n/a	n/a	no	yes
Hypermobility of joints	yes	no	n/a	n/a	n/a	n/a
Radial head dislocation	yes	no	n/a	n/a	yes	n/a
Intelligence	nl	nl	nl	nl	n/a	nl

DXA, dual-energy X-ray absorptiometry; n/a, not available; nl, normal

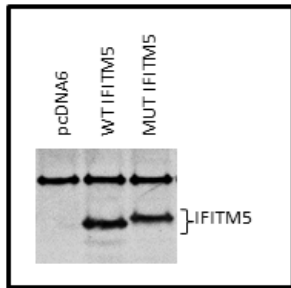


Figure 2. Expression of wild-type (WT) and mutant (MUT) IFITM5 protein. HEK293 cells were transiently transfected with empty vector (pcDNA6), WT IFITM5, or MUT IFITM5 cDNA constructs tagged with the V5 epitope. Cells were lysed 48 hours later and proteins separated by SDS-PAGE followed by Western blot using an anti-V5 antibody. MUT IFITM5 migrates slower than WT IFITM5 due to the presence of the 5 additional amino acids at the N-terminus.

intracellular face of the cell membrane.¹⁹ In addition, it is not clear whether IFITM5 exists as a cell surface molecule, resides within the membranes of the secretory pathway, or shuttles between both.¹⁷ Presumably, the additional 5 amino acids disturb interactions critical for normal bone development. The only currently known interaction partner of IFITM5 is the chaperone FKBP11.^{18,20} While a functional connection between FKBP11 and collagen has yet to be demonstrated, mutations in the related family member FKBP10 cause OI type XI.²¹ Thus, one promising line of investigation will be to determine whether the mutated IFITM5 protein impacts FKBP11 function and whether type I collagen synthesis or assembly is impaired as a consequence.

CONCLUSIONS

We report 7 individuals with OI type V from a wide ethnic background including white, Hispanic, and African/African-American individuals with the canonical IFITM5 mutation. All patients exhibited significant clinical variability, suggesting that genetic background and modifier genes contribute to the variable phenotype. Because 1 patient presented with features characteristic of “classic” type I OI, we suggest that the IFITM5 mutation should be considered in all OI types.

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