SHORT REPORT

Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.–14C>T mutation in all patients

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ABSTRACT

Background Osteogenesis imperfecta (OI) type V is an autosomal dominant bone fragility disorder that we had described a decade ago. Recent research has shown that OI type V is caused by a recurrent c.–14C>T mutation in IFITM5. In the present study, we assessed all patients diagnosed with OI type V at our institutions for the presence of the IFITM5 mutation.

Methods IFITM exon 1 was analysed by Sanger sequencing in genomic DNA from 42 patients with OI type V (age: 2–67 years; 18 female).

Results The c.–14C>T mutation of IFITM5 was detected in all individuals. Indicators of disease severity varied widely: Height z-scores (n=38) ranged from −8.7 to −0.1, median −3.5. Median final height was 147 cm in men (N=15) and 145 cm in women (N=10). Lumbar spine areal bone mineral density z-scores in the absence of bisphosphonate treatment (n=29) were between −7.7 and −0.7, median −5.3. Scoliosis was present in 57%, vertebral compression fractures in 90% of patients.

Conclusions Even though the disease-causing mutation is identical among patients with OI type V, the interindividual phenotypic variability is considerable.

INTRODUCTION

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder that is mainly characterised by bone fragility and often short stature. Extraskeletal findings, such as tooth abnormalities (dentinogenesis imperfecta), and blue or gray sclera can be associated.1 OI is usually transmitted in an autosomal dominant fashion and is mostly caused by mutations in COL1A1 and COL1A2, the genes encoding collagen type I α-chains. Clinically, such patients are classified into OI types I to IV.2 Recessively inherited OI is rare and can be caused by mutations in at least six different genes.3

In 2000 we described an autosomal dominant bone fragility disorder that we called OI type V (Mendelian Inheritance in Man [MIM] 610967).4 OI type V resembles OI type IV with regard to fracture incidence, long-bone deformities, vertebral compression fractures and scoliosis.5 Distinguishing clinical features of OI type V are hyperplastic callus formation after some fractures, calcification of the interosseous forearm membrane, and a mesh-like lamellation pattern on bone histology.4 6 Patients with OI type V do not have blue sclera or dentinogenesis imperfecta.

Recent research has shown that OI type V is caused by a recurrent heterozygous mutation in IFITM5, which encodes BRIL, a protein with unknown function that is specifically expressed in the skeleton.7 8 The mutation consists of a C>T transition at position −14 of the 5’ untranslated region (UTR) of IFITM5, leading to the addition of five amino acids at the N-terminus of the protein. Until now, 19 Korean OI type V patients from nine different families and two German patients from two different families have been reported to have this mutation.7 8

In the present study, we assessed the largest group of OI type V patients reported to date for the IFITM5 mutation, including all patients that had been included in the original description4 and our subsequent reports on the disorder.5 6 10 11 We also provide additional phenotypic information on OI type V.

PATIENTS AND METHODS

The study population comprised 42 subjects with OI type V (age at last follow up: 2–67 years; 18 female; 24 male) who were members of 23 different families. The cohort included all 33 individuals from 21 families who were diagnosed with OI type V at the Shriners Hospital for Children in Montreal between 1995 and 2011. The other two families were recruited through international collaborations. Study participants represented a range of ethnic origins (French Canadian, Hungarian, Belgian, German, Danish, English, Irish, Greek, Israeli, Kuwaiti, Chinese, Peruvian, Puerto Rican).

In each family, at least one member fulfilled our diagnostic criteria for OI type V, namely a history of low trauma fractures and at least one of the following: (1) mesh-like pattern of lamellation under polarised light microscopy of iliac bone samples, (2) history of hyperlastic callus formation, (3) calcification of the interosseous membrane of the forearm.4 Clinical information was extracted from medical charts. The methodologies used for clinical assessments are described in the online supplementary data. The study was approved by the Institutional Review Board of McGill University. Informed consent was provided by participants or, for minors, their parents. Assent was provided by participants aged 7–17 years.
Genomic DNA was extracted from blood or saliva samples. In the first phase of the project, whole-exome sequencing was performed on DNA from five families, using the SureSelect Human Exome Kit V3 (Agilent Technologies, Inc., Santa Clara, California, USA) and Illumina HiSeq2000. Exome sequencing data were analysed, as described. Briefly, Burrows-Wheeler Aligner (V0.5.9) was used to align high quality trimmed paired-end reads against human reference genome (hg19). On average, 86% (+1.36%) of the bases in consensus coding sequence exons were covered by at least 20 reads.

Following the description of a recurrent IFITM5 mutation as a cause of OI type V, we assessed the available DNA samples from individuals with OI type V using Sanger sequencing (see online supplementary table S1). Exon 1 of IFITM5 was amplified by PCR and analysed by Big Dye Terminator cycle sequencing on an Applied Biosystems 3100 DNA sequencer. Results were compared with the IFITM5 reference sequence (RefSeq NM_001025295.1).

To assess a possible functional interaction of the IFITM5 mutation with another pathway known to cause an OI phenotype, we evaluated serum levels of pigment-epithelium derived factor, the protein affected in OI type VI, using an ELISA as described. Results were compared with the IFITM5 reference sequence (RefSeq NM_001025295.1).

RESULTS

We recruited 42 participants with OI type V, representing 9 families with 28 affected members and 14 simplex individuals. The available clinical information is summarised in table 1.

Exome sequencing in patients from five different families did not reveal any mutations in genes that were known to be associated with OI. In addition, the comparison of identified variants in all affected individuals among five families did not lead to the identification of a common candidate gene. However, Sanger sequencing of IFITM5 exon 1 revealed that all 42 participants had the C>T transition at position c.−14 of IFITM5 that had previously been described in OI type V.

Review of the available radiographic documentation revealed that the majority of patients had at least one episode of typical hyperplastic callus formation following fracture, as previously reported (figure 1A, table 1). Periosteal bone proliferation was also observed at skeletal sites that had not been fractured before (figure 1B–D). Forearm radiographs showed some degree of calcification of the interosseous membrane in all but one patient (figure 1E). However, cross-sectional images of the forearm obtained by peripheral quantitative CT revealed that the formation of new ossified tissue was not always limited to the area of the interosseous membrane (figure 1F). Spine radiographs revealed abnormalities (vertebral compression fractures and/or scoliosis) in most patients (figure 1G, table 1).

Height z-scores ranged between −8.7 and −0.1 (median −3.5) and were higher in patients who had completed at least 1 year of bisphosphonate treatment before the age of 13 years (figure 1H, table 1). Median final height was 147 cm in men (N=15) and 145 cm in women (N=10).

The wide interindividual variability in disease severity reflected in the large range of height z-scores was also observed within families. For example, in one family an affected man had been able to walk independently until early adulthood. Two of his children had severe bone fragility and died in early childhood from pulmonary complications. Two affected sons never acquired independent mobility and had severe short stature (final height z-score −7). Another affected son was able to ambulate independently indoors and reached a final height z-score of −2.8.

In the entire cohort of patients with OI type V, lumbar spine areal bone mineral density (BMD) z-scores (in the absence of bisphosphonate treatment) ranged from −7.7 to −0.7, median −5.3 (figure 1I). The mean areal BMD z-score was significantly lower in male than in female patients (figure 1I).

Mean serum levels of pigment-epithelium derived factor were similar between patients with the IFITM5 mutation and OI patients with mutations in COL1A1/COL1A2 (13.3 mg/l (SD 4.9) vs 10.4 mg/l (SD 4.8); p=0.55; t test).

DISCUSSION

In this study we found that all of our patients with a clinical diagnosis of OI type V were positive for a previously described 5′UTR mutation in IFITM5. It thus appears that there is little, if any, genotypic variability among patients with OI type V, even though there is considerable phenotypic variability.

The clinical diagnosis of OI type V was based on the presence of bone fragility and at least one of the findings that distinguish OI type V from other OI types (mesh-like lamellation pattern on bone histology, calcification of the interosseous membrane of the forearm, history of hyperplastic callus formation). As previously reported, mesh-like bone lamellation was present in all 36 patients who had undergone iliac bone biopsy, between 21 months and 15 years of age (table 1). However, bone biopsy is invasive and therefore is not practical for routine diagnosis. Calcification of the interosseous membrane was present in 22 of 23 patients aged 4 years and older. Interosseous membrane calcification seems to develop only after the 1st months of life and therefore can be absent in young babies. Similarly, we have not observed hyperplastic callus formation before the age of 9 months, whereas 14 of 17 (82%) patients aged 14 years and older had at least one episode of hyperplastic callus formation.

Given the lack of specific clinical findings for OI type V in young babies and the ease of diagnosing OI type V by sequence analysis of IFITM5, it may be an efficient approach to perform IFITM5 sequence analysis as a first test in the molecular diagnostic workup of young babies who have suspected OI and a negative family history for OI. In older subjects with suspected OI...
who do not have either scleral discoloration nor dentinogenesis imperfecta, performing IFITM5 analysis as a first test may also be an efficient strategy.

The phenotypic variability in OI type V is highlighted by the wide range of height and BMD results in this patient group. Short stature in OI type V may to some degree be the consequence of bone fragility, as suggested by the observation that bisphosphonate treatment during the growing years was associated with higher height z-scores. However, it is also possible that the disease-causing mutation has a direct effect on growth plate activity, as IFITM5 is expressed in the structures surrounding the growth plate. In younger children with OI type V, the growth plate can have a widened and irregular aspect and a hyperdense metaphyseal band is often visible adjacent to the growth plate. It appears that some of the phenotypic variability is due to gender differences, as males tended to have larger deficits in height and BMD, but other and as yet unidentified factors clearly play an important role. These observations indicate that even though the diagnosis of OI type V is now easy to establish by IFITM5 sequencing, it is still difficult to make a prognosis regarding disease severity, given the wide phenotypic variability associated with this mutation.

In the initial phase of this project, the genetic defect underlying OI type V was still unknown and we analysed genomic

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**Figure 1** Characteristics of osteogenesis imperfecta (OI) type V caused by the IFITM5 c.−14 C>T mutation. (A) Typical hyperplastic callus at the femur (asterisk). (B–D) Periosteal new bone formation at sites where no fractures had occurred before (indicated by arrows). Femur (B); fibula (C); metacarpals and distal ulna (D). Hyperdense bands immediately adjacent to the growth plates are also visible in (D), as well as metaphyseal lines due to prior bisphosphonate treatment. (E) Calcification of the interosseous membrane of the forearm. (F) Cross-sectional images of the shaft of the radius and ulna by peripheral quantitative CT in a control subject (upper panel) and three patients with OI type V (three lower panels). In the patients, sites of periosteal new bone formation are evident at several locations (circled by dashed lines), leading almost to a fusion of radius and ulna in the example shown in the lowest panel. (G) Antero-posterior view of the thoracic spine and ribs, showing thin and wavy ribs as well as compression fractures of all vertebrae. (H) Height z-scores. Full symbols indicate patients who completed at least 1 year of bisphosphonate treatment before the age of 13 years. The bar graphs indicate mean and SE. BP, bisphosphonate; TX, treatment. (I) Last lumbar spine areal BMD before the start of bisphosphonate treatment or last available result in subjects who have not had bisphosphonates. The bar graphs indicate mean and SE.

DNA samples from five families with OI type V by whole-exome sequencing. This did not lead to the discovery of the disease-causing mutation, for two reasons. First, only coding mutations in annotated protein coding sequences but not UTRs were considered. Second, the capture for exon 1 of IFITM5 was very poor and the capture did not allow calling variants in that exon.

How the addition of five amino acids to the N-terminus of IFITM5 leads to the clinical picture of OI type V is unclear at present, even though it is known that IFITM5 is highly expressed in osteoblasts. We did not find evidence for an abnormality in the regulation of pigment-epithelium derived factor, which is affected in OI type VI. Our previous bone histomorphometric studies in patients with OI type V have demonstrated a bone formation defect of trabecular osteoblasts, similar to OI caused by COL1A1/COL1A2 mutations. This trabecular bone formation deficit may account for the low bone mass and bone fragility that is common to all types of OI.

The findings that are specific for OI type V (hyperplastic callus formation, calcification of the interosseous membrane of the forearm) involve the outer surface of bones. Our present observations suggest that the so-called calcification of the interosseous membrane is not an ectopic calcification but rather represents periosteal proliferation and new bone formation that may use the interosseous membrane as a guiding structure. Overall, it appears that the IFITM5 mutation leads to a dysregulation of periosteal bone formation in addition to the bone formation deficit in trabecular bone.

In conclusion, this study demonstrates the presence of a recurrent IFITM5 mutation in a relatively large population of individuals with OI type V. Even though the disease-causing mutation is identical among patients, the interindividual phenotypic variability is considerable.

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Contributors All authors contributed to the concept, design, acquisition of data or analysis, interpretation of data, drafting or revising the content and approved the final version.

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REFERENCES
Osteogenesis Imperfecta Type V: Marked Phenotypic Variability Despite the Presence of the \textit{IFITM5} c.-14C>T Mutation in all Patients

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Supplemental Data

Supplemental Materials and Methods

\textbf{Anthropometry.} Height was measured using a Harpenden stadiometer (Holtain, Crymych, UK). Height and weight measurements were converted to age- and sex specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention.[19] For each individual, the last available height and weight data were recorded for this study. Patients were assumed to have reached final height if they had not grown for one year or when they had reached 16 years of age for females or 18 years for males.

\textbf{Relationship Between Bisphosphonate Treatment and Height.} We compared patients who had received at least one year of bisphosphonate treatment before they had reached 13 years of age with patients who had not received at least one year of bisphosphonate treatment before 13 years of age. The age of 13 years was taken as a cut-off in this analysis as bisphosphonate treatment given at the end of the growth period or after the completion of growth is unlikely to have a major influence on height.

\textbf{Bone Densitometry.} Dual-energy X-ray absorptiometry was performed in 30 subjects. Measurements were obtained in the anterior–posterior direction at the lumbar spine (L1–L4)
using a Hologic QDR Discovery device (Hologic Inc., Waltham, MA, USA). Lumbar spine areal BMD results were transformed to age-specific z-scores using reference data provided by the densitometer manufacturer.[20, 21] The last available areal BMD z-score in the absence of bisphosphonate treatment was recorded for this study. BMD measures taken after the first bisphosphonate exposure were excluded, because bisphosphonate treatment has a major effect on this parameter.[5]

**Analysis of Spine Radiographs.** The last available spine radiographs were analyzed of the 30 patients in whom adequate films were available. The antero-posterior radiographs were assessed for scoliosis with a Cobb angle of >20 degrees. The lateral radiographs were evaluated for the presence or absence of vertebral compression fractures. The analyses were performed by a radiologist.

**Statistical Analysis.** As most of the variables were not normally distributed, Mann-Whitney U-tests were used to evaluate group differences in continuous variables. A 5% significance level was maintained throughout these analyses, and all tests were two-sided. Calculations were performed using IBM SPSS Statistics software version 20 for Windows (IBM Inc., Armonk, NY, USA).
Supplemental Table S1. Primers used in this study.

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<th>Exon</th>
<th>Sequence</th>
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<td>1</td>
<td>5’- CCGCAGGCTGTAATTTGTG -3’</td>
<td>371 bp product (used for PCR and sequencing)</td>
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<td></td>
<td>5’- CCACCTTGATGGAGTAGTGG -3’</td>
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