Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

A novel COL1A1 gene-splicing mutation (c.1875+1G>C) in a Brazilian patient with osteogenesis imperfecta

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Genet. Mol. Res. 8 (1): 173-178 (2009)
Received December 4, 2008
Accepted December 10, 2008
Published February 17, 2009

ABSTRACT. Osteogenesis imperfecta is a heterogeneous genetic disorder characterized by bone fragility and deformity, recurrent fractures, blue sclera, short stature, and dentinogenesis imperfecta. Most cases are caused by mutations in COL1A1 and COL1A2 genes. We present a novel splicing mutation in the COL1A1 gene (c.1875+1G>C) in a 16-year-old Brazilian boy diagnosed as a type III osteogenesis imperfecta patient. This splicing mutation and its association with clinical phenotypes will be submitted to the reference database of

COL1A1 mutations, which has no other description of this mutation.

Key words: Osteogenesis imperfecta type III; Splicing mutation; Genotype/phenotype correlation

INTRODUCTION

Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder characterized by bone fragility and deformity, recurrent fractures, blue sclera, short stature, and dentinogenesis imperfecta. Based on clinical and radiological findings, OI is traditionally subclassified as type I (mild), II (lethal), III (severe), and IV (moderate), but recent classifications include types V, VI and VII, based on histological and radiological aspects (Sillence et al., 1979; Glorieux et al., 2000, 2002; Ward et al., 2002; Barnes et al., 2006).

Most cases are caused by mutations with dominant inheritance in COL1A1 and COL1A2 genes, which code for type I collagen chains, an important structural protein of bone, cartilage and tendons (Barsh and Byers, 1981; Gajko-Galicka, 2002; Marini et al., 2007). However, there are rare cases of recessive OI associated with mutations in the CRTAP and LEPRE1 genes (Morello et al., 2006; Cabral et al., 2007).

In general, mutations in COL1A1 and COL1A2 genes cause quantitative or qualitative defects, associated with mild and severe forms of OI, respectively (Redford-Badwal et al., 1996; Forlino and Marini, 2000). Nonetheless, the genotype/phenotype correlation is not completely established and molecular studies associated with clinical investigations are necessary to better understand the genetic aspects of OI.

We present a novel mutation in the COL1A1 gene of a proband diagnosed as a type III OI patient, the most severe non-lethal form of OI.

SUBJECT AND METHODS

This study was approved without restrictions by the Research Ethics Committee of the Hospital Infantil Nossa Senhora da Glória, and authorized by the patient's mother.

Clinical report

The patient is a 16-year-old Brazilian boy diagnosed with type III OI (severe), based on clinical aspects. He is one of two sons of a non-consanguineous couple, and he is the only diagnosed case in his family. At birth, he had several fractures and at the time of the study, he had suffered over 100 fractures. Currently, the patient is approximately 121 cm tall and weighs 32 kg. His head circumference is 60 cm, which is below the 2.5% percentile. The fractures have decreased and he does not have intramedullary rods. However, the limbs are seriously deformed and he has needed a wheelchair for mobility since 8 years old. Hearing is normal, but the patient has a blue sclera and myopia. He has dentinogenesis imperfecta and osteopenia. The radiographs show a rectification of the thoracolumbar spinal column and the attenuation of the kyphosis and lordosis. The changes in bone mineral density of the lumbar spine (L1-L4) and total body during the treatment with pamidronate can be seen in Figures 1 and 2. The patient has been treated with pamidronate since he was 11 years old.

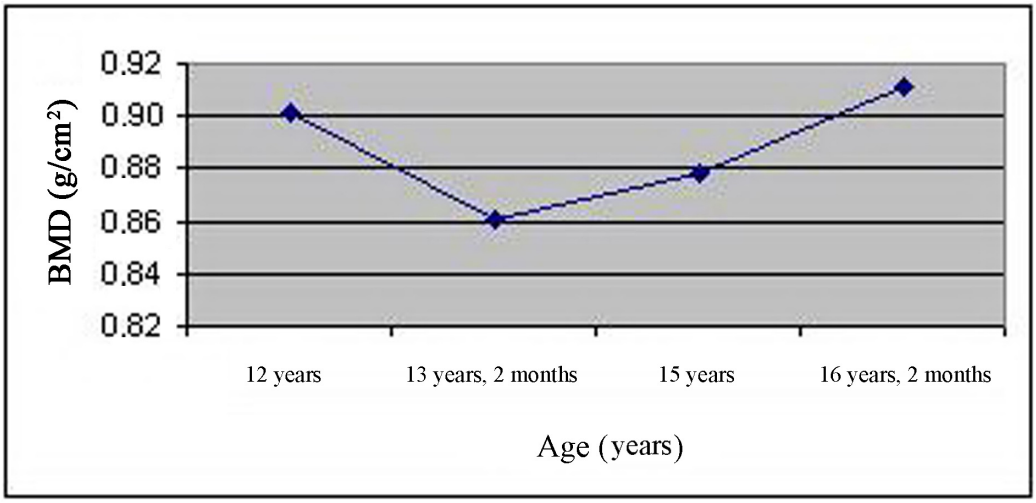

Figure 1. Bone mineral density, BMD (total body) in g/cm^2 during treatment with pamidronate.

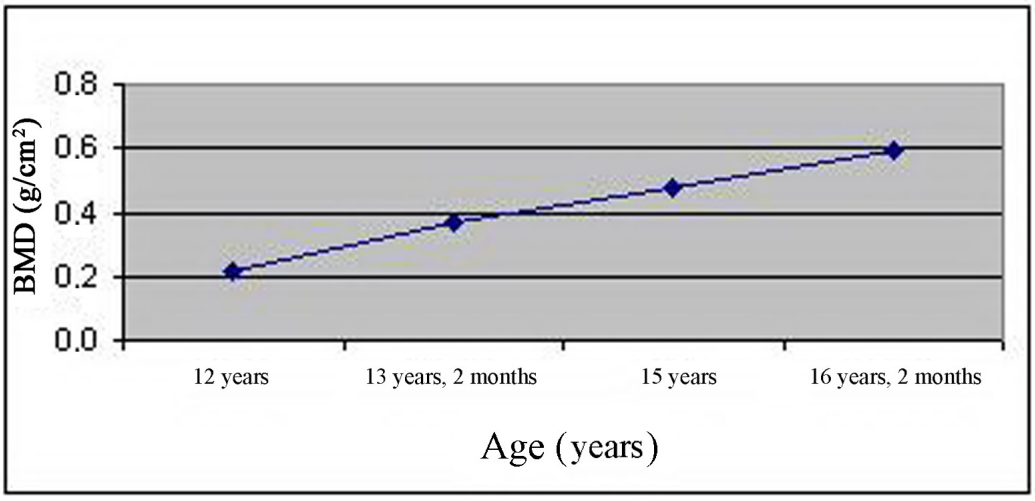

Figure 2. Bone mineral density, BMD (lumbar spine L1-L4) in g/cm^2 during treatment with pamidronate.

Molecular analysis

The proband's DNA, as well as DNA of his mother and one hundred unaffected individuals of the same population, were extracted from peripheral blood cells (Miller et al., 1988). It was not possible to analyze the proband's father's DNA because he was deceased. Fragments containing the exons of the COL1A1 gene were amplified in an Applied Biosystems GeneAmp®PCR System 9700 model using primers described by Körkkö et al. (1998). Poly-

merase chain reaction products were screened for mutations by single-stranded conformation polymorphism, on 5% polyacrylamide-5% glycerol gels and on 6% glycerol MDE (Cambrex Bio Science Rockland, Inc.) gels (Orita et al., 1989; Spinardi et al., 1991). Fragments showing abnormal migration pattern were sequenced to detect variation in the DNA sequences, which were subsequently compared with mutations described in the Osteogenesis Imperfecta Mutation Database (Dalgleish, 2008) and whose reference sequence is Z74615 (NCBI, 2008).

RESULTS

Molecular screening showed abnormal migration patterns for fragments containing exons 27 (Figure 3), 30, 31, 44, and 45. Control samples also showed the same abnormal patterns for exons 30, 31, 44, and 45, but not for exon 27. Therefore, exon 27 of the proband's mother was also analyzed and found to show a normal migration pattern.

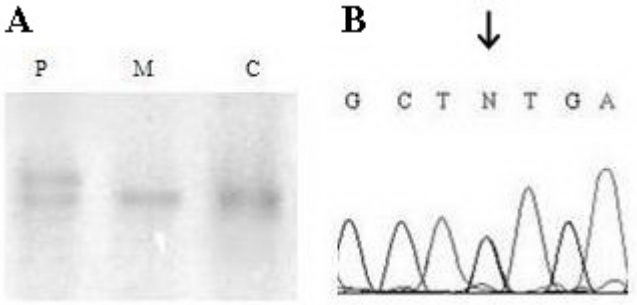

Figure 3. Molecular analysis of the proband's fragment containing exon 27. **A.** Single-stranded conformational polymorphism analysis showing abnormal migration pattern for the proband (P) when compared with the proband's mother (M) and a control (C) sample. **B.** Sequencing of the abnormal fragment resulting in the identification of a guanine to cytosine substitution in intron 27 (c.1875+1G>C).

Sequencing of the abnormal fragments of the patient resulted in the identification of five different nucleotide substitutions (Table 1).

Table 1. Allelic frequency of the COL1A1 nucleotide substitutions detected in the osteogenesis imperfecta patient.

Substitution	Location	Variation	Allelic frequency in 100 control samples
c.1875+1G>C	Intron 27	Splicing mutation	0.00
c.1984-41A>G	Intron 29	Polymorphism	0.08
c.2028+39T>C	Intron 30	Polymorphism	0.13
c.3208-32C>A	Intron 44	Polymorphism	0.05
c.3261+31C>T	Intron 45	Polymorphism	0.10

DISCUSSION

The most notable genetic variation was the substitution of a guanine for a cytosine in the first nucleotide immediately after exon 27, in the splicing site of intron 27 of the patient with type III OI.

Precision in the splicing process is guided by conserved sequences at the 5' and 3'ends of all introns. Dinucleotides GT (on the 5' end) and AG (on the 3' end) on the ends of the introns are needed for correct splicing, and variations in these nucleotides could interfere with the normal synthesis of mRNA (Schwarze et al., 1999). The proband showed a variation in the intron 27 5'-dinucleotide of the COL1A1 gene. We suggest that the guanine to cytosine substitution in one of the intron 27 conserved regions interfered with normal splicing and the mutant intron was not precisely removed, causing OI.

Because the proband's parents were not diagnosed as OI patients and his mother's DNA did not show the mutation c.1875+1G>C, we also suggest that this is a *de novo* mutation case.

The splicing mutation described in this report and its association with clinical phenotypes will be submitted to the reference database of COL1A1 mutations, which has no other description of the mutation reported here (Dalgleish, 2008).

Four of the five variations described here were detected in the patient and in control samples as well, characterizing polymorphic sites. The c.1984-41A>G, c.2028+39T>C, c.3208-32C>A, and c.3261+31C>T substitutions were found in a relatively high number of control samples. We also observed that these variations were single nucleotide substitutions in introns, not resulting in amino acid changes. Thus, because of the frequency in control samples and the location of the variations in the COL1A1 gene (introns 29, 30, 44, and 45), we suggest that these are non-pathogenic polymorphisms.

In conclusion, we detected four COL1A1 gene non-pathogenic polymorphisms and a novel splicing mutation (c.1875+1G>C) associated with type III OI. The genotype/phenotype correlation described here provides new information about the genetic aspects of OI.

ACKNOWLEDGMENTS

We thank the Hospital Infantil Nossa Senhora da Glória for clinical support and the Brazilian institutions ARCELOR MITTAL BRASIL, FAHUCAM, CNPq, and UFES for financial support. C. Barbirato was supported by CNPq and UFES grants. We also thank Marcos Vinícius Dornelas de Moraes, Bruno Vinícius Pimenta de Almada and Geise Quirino for laboratory support.

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