Osteogenesis imperfecta (OI) is a dominant autosomal disorder caused by mutations in type I collagen genes, COL1A1 and COL1A2, which are responsible for synthesis of this main protein of bones, skin, ligaments, tendons and most other connective tissues. Those genes encode the \( \alpha_1 \) and \( \alpha_2 \) chains of the collagen triple helix, respectively (Gajko-Galicka et al. 2002).

OI patients present with bone fragility and skeletal deformity within a broad phenotypic range: from a mild (type I) to a lethal form (type II), including two other types presenting varied severity (types III and IV). The mild forms are usually caused by mutations that inactivate one allele of the COL1A1 gene, resulting in a reduced amount of normal type I collagen. On the other hand, the severe and lethal forms result from dominant negative mutations in COL1A1 or COL1A2, which produce structural defects in the collagen molecule (Gajko-Galicka et al. 2002).

The varied clinical characteristics of OI reflect different classes of mutations in different regions of type I collagen genes. Consequently, more than 250 different mutations in the COL1A1 and COL1A2 genes had been characterized and they have been registered in the Human Type I and Type III Collagen Mutations Database (Dalgleish 1997; http://www.le.ac.uk/genetics/collagen). Besides, these alterations vary in type and location, although the most common in COL1 loci are single-base substitutions in the part of the gene coding for the triple helix domain, which result in replacing glycine by an amino acid with a bulkier side chain.

Summarizing, the main objective of the present study was to search for mutations in the COL1A1 gene in 13 unrelated Brazilian OI patients. This is the first molecular study of OI in Brazil. We found 6 mutations, 4 of them novel (c.1885delG, p.P239A, p.G592S, p.G649D) and 2 previously described (p.R237X and p.G382S). Thus, the findings show that there are no prevalent mutations in our sample, and that their distribution is similar to that reported by other authors, with preponderance of substitutions for glycine in the triple helix domain, causing OI types II, III and IV.

**Key words:** COL1A1, mutation, osteogenesis imperfecta, type I collagen.

Molecular findings in Brazilian patients with *osteogenesis imperfecta*

Fernanda C. Reis\(^1\), Fabiana Alexandrino\(^1\), Carlos E. Steiner\(^2\), Denise Y.J. Norato\(^2\), Denise P. Cavalcanti\(^2\), Edi L. Sartorato\(^1\)

\(^1\) CBMEG/Human Genetics Laboratory and \(^2\) Department of Medical Genetics/FCM, UNICAMP, Campinas, SP, Brazil

**Abstract.** *Osteogenesis imperfecta* (OI) is a genetic disorder of increased bone fragility and low bone mass. Severity varies widely, ranging from intrauterine fractures and perinatal lethality to very mild forms without fractures. Most patients with a clinical diagnosis of OI have a mutation in the *COL1A1* or *COL1A2* genes that encode the \( \alpha_1 \) and \( \alpha_2 \) chains of type I procollagen, the major protein in bones. Hence, the aim of the present study was to identify mutations in the *COL1A1* gene in 13 unrelated Brazilian OI patients. This is the first molecular study of OI in Brazil. We found 6 mutations, 4 of them novel (c.1885delG, p.P239A, p.G592S, p.G649D) and 2 previously described (p.R237X and p.G382S). Thus, the findings show that there are no prevalent mutations in our sample, and that their distribution is similar to that reported by other authors, with preponderance of substitutions for glycine in the triple helix domain, causing OI types II, III and IV.
gene in Brazilian patients previously diagnosed as having OI. Also, we wanted to collect information about the characteristics of mutations in this gene in our sample.

Thirteen patients recruited from different states were diagnosed as having OI types I, II, III or IV, according to their clinical and radiological characteristics, on the basis of the Sillence OI classification (Sillence et al. 1979). Blood samples were obtained with the approval of the Ethics Committee of the Medical Academy and with informed parental consent. Total genomic DNA was extracted from 10 ml of peripheral blood by phenol-chloroform standard methods. All exons of the \( \text{COL1A1} \) gene and its flanking sequences, with the exception of the 6 exons encoding the N-propeptides, were amplified by PCR, followed by direct DNA sequencing. The primers and PCR conditions used were previously described (Körkkö et al. 1998). PCR-amplified segments of the \( \text{COL1A1} \) gene were sequenced using an ABI PRISM™ 377 Sequencer and Big Dye Terminator Sequencing protocol (PE Biosystems Foster City CA, USA), according to the manufacturer’s recommendations. Next, the mutations were identified by a comparison of the obtained DNA sequence with the reference cDNA sequence of the \( \text{COL1A1} \) gene (GenBank accession number Z74615).

Six different mutations in the \( \text{COL1A1} \) gene were identified. Five of them were from unrelated, sporadic cases and one was from a familial case. Four of the 6 mutations are novel (i.e. not yet registered in the Human Type I and Type III Collagen Mutations Database). Among them, 3 are missense mutations (p. P239A, p. G592S and p. G649D) and one is a single-base deletion (c. 1885delG). The characteristics of these mutations and information on their clinical manifestation are summarized in Table 1.

The most frequent mutations that change a codon for glycine in the triple helical domain in

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Mutation</th>
<th>Exon OI type</th>
<th>Blue sclerae</th>
<th>Dentinogenesis imperfecta</th>
<th>Hearing loss</th>
<th>Fractures at birth</th>
<th>Multiple fractures at birth</th>
<th>Bone deformity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>c.2308G&gt;A</td>
<td>p.G592S</td>
<td>33/34 III</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>77</td>
<td>c.2480G&gt;A</td>
<td>p.G649D</td>
<td>37 IIA</td>
<td>+</td>
<td>/</td>
<td>/</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>43</td>
<td>c.1249C&gt;G</td>
<td>p.P239A</td>
<td>19 I</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>28</td>
<td>c.1885delG</td>
<td>frameshift at G451</td>
<td>28 I</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>this study</td>
</tr>
</tbody>
</table>

Features: (+) denotes presence; (–) denotes absence, (/) denotes non corresponding and (?) denotes unknown.

either of the two alleles of \( \text{COL1A1} \) or \( \text{COL1A2} \) are responsible for the severe variants of the disease OI types II, III and IV (Culbert et al. 1996). In this study, three of these single-nucleotide substitutions in the \( \text{COL1A1} \) gene were identified: p.G382S, p.G592S, and p.G649D (Table 1, patients 74, 33 and 77, respectively).

As described before by Mackay et al. (1993), the mutation c.1678G>A (p.G382S) was found in a patient diagnosed as having OI type IV (Figure 1, patient 74). Also, a second mutation was identified at that position, although the residue changed was not serine but cysteine, resulting in a mild OI type IV (Byers 1990). This shows the relevance of the nature of the residue that substitutes for glycine in determining the phenotype (Mackay et al. 1993).

The c.2308G>A mutation also resulted from a substitution of a glycine residue by serine (p.G592S), causing a severe OI type III (Figure 1, patient 33). The correlation between the positions of serine substitutions with the severity of the OI phenotype is interesting, since they do not show
a gradient of severity that depends on the position of the mutation on the triple helix (Byers 1990). Thus, the mutations reported here reinforce this hypothesis because substitutions at positions 382 and 592 resulted in non-lethal phenotypes of OI type IV and severe OI type III, respectively.

The structural alteration c.2480G>A (p.G649D) was identified in an OI type IIa patient, which leads to a lethal phenotype (Figure 1, patient 77). Taking into account the genotype-to-phenotype correlations, the lethal outcome of this mutation is not surprising, since all the substitutions of glycine by the bulky aspartic acid in any position along the α1(I) collagen chains have so far been described as lethal, basing on the Human Type I Collagen Mutations Database.

The most unusual mutation found in this OI population was p.P239A (Table 1 and Figure 1, patient 43). This point mutation converts proline to alanine, because of a change in nucleotide 1249 of the COL1A1 gene in a patient with OI type I phenotype. Proline substitutions for alanine are very rare and this is the first one described in a patient with low bone density but no apparent metabolic bone disease. The substitution identified before by Spotila et al. (1994) was c. 613C>G (p. P27A), and its effect on the biological function of type I collagen, as well as its role in osteopenia, is uncertain. Since the c.1249C>G was the only alteration identified in the COL1A1 coding sequence of that patient and the sequence was situated in the middle of the triple helix domain, Berg and Prockop (1973) suggested that proline and hydroxyproline promote triple helix stability. Thus alterations in those amino acid residues may be responsible for mild OI phenotypes. This patient has two affected brothers but his parents are apparently normal in respect of OI clinical symptoms. The recurrence of their phenotype may be explained by parental mosaicism that results in affected children from unaffected parents.

Most of mutations found in patients with OI type I could introduce either premature-termination codons for translation or aberrant RNA splicing. Moreover, they thereby reduce the expression of the COL1A1 gene. Mutations introducing a premature stop codon are the most frequent cause for a null COL1A1 allele arising either directly from a point mutation or indirectly from
a frameshift mutation (Körkkö et al. 1998). As such, two COL1A1 gene mutations identified in this study were considered as null mutations: c.1885delG and p.R237X (Table 1, patients 28 and 4).

The frameshift mutation at position 1885 of COL1A1 was found in a patient with a very mild OI type I (Figure 1, patient 28). Single-base deletions are relatively rare in type I collagen genes, but many of them have already been described as causing OI type I. They tend to occur in the common sequence context of CCC CCT (Körkkö et al. 1998), although this is not a characteristic of the deletion found in this study.

The nonsense mutation p.R237X converts a codon for arginine to a premature stop codon in nucleotide 1243, producing a truncated protein (Figure 1, patient 4). As previously reported by Körkkö et al. (1998), the majority of the mutations that converted the arginine codon CGA to the premature stop codon TGA was found in the sequence context of G/CCC CGA GG/T in the COL1A1 gene. The p.R237X identified in this study is in such a sequence (GCC CGA GG). This mutation was found in a patient who had been clinically diagnosed as having OI type IV, but other cases of this same alteration are described in patients with OI type I (Redford-Badwal et al. 1996, Willing et al. 1996). Variable phenotypic outcomes were observed in unrelated cases bearing the same mutation also by Zhuang et al. (1996).

No mutations were found in the remaining 7 patients, even though they present the typical clinical features of OI. Probably these patients have mutations on the COL1A2 gene, which was not analyzed in this study but is equally involved with this disease.

Finally, as expected, there are no predominant mutations in Brazilian OI patients in the COL1A1 gene. The lack of a small cluster of mutations in OI, combined with the fact that mutations are scattered throughout the gene and their private nature in each family, makes mutational analysis an expensive and labor-intensive process. Anyway, the description of novel mutations and their correlation with phenotype may definitely contribute to the disease prognosis.

Acknowledgements. This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), and by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

References


