Relationship Between Genotype and Skeletal Phenotype in Children and Adolescents with Osteogenesis Imperfecta

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ABSTRACT
Osteogenesis imperfecta (OI) is a heritable bone fragility disorder that in the majority of cases is caused by mutations in COL1A1 or COL1A2, the genes that encode the two collagen type I \( \alpha \) chains, \( \alpha1(I) \) and \( \alpha2(I) \). In this study, we examined the relationship between collagen type I mutations and bone densitometric and histomorphometric findings in pediatric OI patients who had not received bisphosphonate treatment. Lumbar spine areal bone mineral density (LS aBMD) was measured in 192 patients (99 girls, 93 boys; age range 3 weeks to 16.9 years) who had either COL1A1 mutations leading to haploinsufficiency \( (n = 52) \) or mutations that lead to the substitution of glycine by another amino acid in the triple-helical domain of either the \( \alpha1(I) \) \( (n = 58) \) or the \( \alpha2(I) \) chain \( (n = 82) \). Compared with patients with helical mutations, patients with COL1A1 haploinsufficiency on average were taller and heavier and had higher LS aBMD. After adjustment for age, sex, and height Z-scores, the mean LS aBMD Z-scores were \(-4.0\) for the haploinsufficiency group and \(-4.7\) for both helical mutation groups. In the whole patient population, the average LS aBMD Z-score was higher by 0.6 (95% confidence interval 0.2–1.0) in girls than in boys. Iliac bone histomorphometry (in a subgroup of 96 patients) showed that outer bone size (core width) and trabecular bone volume were similar between genotypic groups, but cortical width was 49% higher in the haploinsufficiency group compared with patients with helical mutations in \( \alpha2(I) \). Bone turnover parameters were lower in the haploinsufficiency group than in patients with helical mutations. In the group of patients with helical mutations, neither the type of \( \alpha \) chain affected, nor the type of amino acid substituting for glycine, nor the position of the mutation in the \( \alpha \) chain had a detectable relationship with LS aBMD or histomorphometric results. Thus patients with haploinsufficiency mutations had a milder skeletal phenotype than patients with mutations affecting glycine residues, but there was no clear genotype-phenotype correlation among patients with helical glycine mutations. © 2010 American Society for Bone and Mineral Research.

KEY WORDS: BONE HISTOMORPHOMETRY; BONE MINERAL DENSITY; COLLAGEN TYPE I; OSTEOGENESIS IMPERFECTA

Introduction
Osteogenesis imperfecta (OI) is a heritable skeletal disorder characterized by bone fragility and often short stature. Most individuals with a clinical diagnosis of OI have an identifiable mutation in COL1A1 or COL1A2, the genes that encode the two collagen type I \( \alpha \) chains, \( \alpha1(I) \) and \( \alpha2(I) \).(1)

The clinical severity of OI ranges from perinatal lethality to nearly asymptomatic individuals. OI patients with collagen type I mutations can be classified into four clinically defined types.(1) OI type I comprises patients with absence of bone deformities and normal or near-normal stature. OI type II is lethal in the perinatal period. OI type III is the most severe form in children surviving the neonatal period and leads to extreme short stature. Patients with mild to moderate bone deformities and variable short stature are classified as OI type IV. Extraskeletal findings, such as tooth abnormalities (dentinogenesis imperfecta), blue or gray color of sclerae, and in adult years, hearing loss can be associated to a variable degree.

The \( \alpha1(I) \) and \( \alpha2(I) \) chains of collagen type I both contain a central triple-helical domain of 1014 amino acids. These triple-helical domains are composed of uninterrupted repeats of the Gly-X-Y tripeptide. Two \( \alpha1(I) \) and one \( \alpha2(I) \) chains intertwine in their helical domains to form a mature collagen type I molecule. Triple-helix formation can proceed normally only if a glycine residue is present in every third position of the chains because glycine is the only residue small enough to fit into the restricted space at the inside of the helix.

There are two general classes of mutations in type I collagen that result in OI. The first are mutations that cause a failure to...
synthesize the products of one COL1A1 allele and thus lead to haploinsufficiency. Three mechanisms are involved: frame shifts owing to small insertions or deletions, point mutations that create termination codons, and some splice-site mutations. These mutations initiate nonsense-mediated decay of the mRNA derived from that allele. Haploinsufficiency mutations consistently result in a clinical picture of OI type I with mild bone fragility.

The second class of mutations includes those that result in the synthesis of collagen molecules with structural abnormalities. This is caused most frequently by the substitution of glycine by another amino acid in the triple-helical domain of either the $\alpha 1(I)$ or the $\alpha 2(I)$ chain. More than 700 different helical glycine mutations have been identified in OI. For most of these mutations, the description of the associated phenotype is limited to OI type and whether or not the affected individuals survived the neonatal period. Nevertheless, it appears from the available information that helical glycine mutations can lead to the whole spectrum of clinical severity of OI from mild OI type I to lethal OI type II. The clinical severity caused by a mutation is thought to depend on the type of $\alpha$ chain affected, the type of amino acid substituted for glycine, and the position of the mutation within the $\alpha$ chain.

Many studies on individual OI patients or small patient groups have examined the molecular and cellular consequences of collagen type I mutations, especially in skin fibroblasts. However, very little is known about how specific collagen type I mutations affect skeletal development at the tissue and whole-bone levels. We have previously presented iliac bone histomorphometric findings in the clinically defined OI types I, III, and IV, but collagen type I sequencing results were not available at the time. With regard to the whole-bone level, Lund and colleagues correlated the results of bone densitometry with collagen type I defects. However, that study included only 16 pediatric OI patients, and the type of mutation could not be defined with precision because collagen defects were examined by protein electrophoresis only.

In this study, we therefore aimed at providing a more detailed picture of the relationship between collagen type I mutations, as assessed by DNA sequence analysis of the COL1A1 and COL1A2 genes, and bone histomorphometric and densitometric findings.

Subjects and Methods

Subjects

The study population is comprised of patients who were evaluated at the Shriners Hospital for Children in Montreal. Patients were assessed clinically by one of the authors (FR or FHG). When a diagnosis of OI was considered likely on clinical grounds, genomic DNA was obtained for sequence analysis of the COL1A1 and COL1A2 genes. Owing to the study setting in a specialized pediatric orthopedic hospital, the study population is comprised exclusively of patients who had survived the immediate postnatal period. This analysis therefore includes patients who were diagnosed as having OI type I, III, or IV according to the Sillence classification but not patients with the most severe phenotype (OI type II).

COL1A1 and COL1A2 mutations were identified in 274 pediatric OI patients (OI type I, $n = 114$; OI type III, $n = 65$; and OI type IV, $n = 95$). This report includes patients in whom DNA sequence analysis demonstrated the presence of either a mutation in COL1A1 leading to haploinsufficiency or a mutation in COL1A1 or COL1A2 leading to the substitution of a glycine residue in the helical domains of the $\alpha 1(I)$ or $\alpha 2(I)$ chains by another amino acid. Mutations in COL1A1 causing frameshifts or point mutations creating termination codons were predicted to lead to haploinsufficiency. The effect of splice-site mutations on mRNA processing is often complex and cannot be predicted on the basis of DNA analysis alone. Therefore, the 44 patients with splice-site mutations (OI type I, $n = 29$; OI type III, $n = 4$; and OI type IV, $n = 11$) were not included in this study. The 19 patients with rarer types of mutations (OI type I, $n = 3$; OI type III, $n = 3$; and OI type IV, $n = 13$), such as propeptide mutations or in-frame deletions, also were excluded because patient numbers were insufficient for statistical analysis.

This analysis includes all patients with haploinsufficiency or helical glycine mutations who had undergone at least one lumbar spine areal bone mineral density (LS aBMD) examination in the absence of prior bisphosphonate treatment. Patients who had such mutations but in whom LS aBMD could not be measured for technical reasons (owing to prior spinal surgery, $n = 2$; OI type III, $n = 1$; or OI type IV, $n = 1$) or who had received bisphosphonate treatment before their first evaluation at our institution ($n = 17$; OI type I, $n = 4$; OI type III, $n = 11$; or OI type IV, $n = 2$) were excluded from the analysis.

Thus 192 patients were included in the analysis of the relationship between genotype and LS aBMD. In addition to bone densitometry, iliac bone biopsies had been performed in a subset of 96 patients either for diagnostic reasons or as a baseline measure in bisphosphonate treatment studies. The clinical characteristics of study participants, as well as their bone densitometric and histomorphometric results, were extracted from their medical records by retrospective chart review. The study was approved by the Shriners Hospital Institutional Review Board. Informed consent was obtained from the legal guardians and/or patients.

Collagen type I mutation analysis

Total genomic DNA was isolated from peripheral blood or saliva using standard extraction methods. All 51 exons of the COL1A1 gene and all 52 exons of the COL1A2 gene, including the exon-intron boundaries, were amplified by polymerase chain reaction (PCR) using primers described previously. The sequencing reaction was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), and the nucleotide sequence was determined using an Applied Biosystems 3100 DNA sequencer.

Sequence traces were aligned with the GenBank reference sequences of the COL1A1 genomic DNA (AF017178) and cDNA (NM_000088.3) and the COL1A2 genomic DNA (AF004877.1) and cDNA (NM_000089.3). Helical mutations were numbered according to the position of the mutated amino acid in the triple helix of each $\alpha$ chain. These triple-helical domains correspond to the residues encoded by codons 179 to 1192 of the COL1A1
transcript and codons 91 to 1104 of COL1A2, when expressed following the convention (www.hgvs.org/mutnomen/recs.html), which starts with the translation initiator methionine as amino acid +1 and the A of the ATG codon as nucleotide +1. The mutations included in this report have been entered into a database of OI mutations (www.le.ac.uk/ge/collagen/).(4,5)

Bone densitometry

Bone densitometry was performed in the anteroposterior direction at the lumbar spine (L1–L4) by dual-energy X-ray absorptiometry (DXA; QDR Discovery, Hologic, Inc., Waltham, MA, USA). A quality control program was conducted throughout the study. LS aBMD results were converted to age- and sex-specific Z-scores combining reference data from Salle and colleagues (for patients younger than 2 years of age) and data provided by the densitometer manufacturer. The manufacturer data were based on the studies of Glastre and colleagues(11) and Southard and colleagues,(12) which were comprised of a total of 353 children and adolescents aged 1 to 19 years.

None of the patients included in this study had received bisphosphonate treatment prior to the densitometric measurement presented here. In patients who never received bisphosphonate treatment, the last available LS aBMD result was used for this analysis. In patients who were treated with bisphosphonates, the last LS aBMD before the start of bisphosphonate therapy was used.

Histomorphometry

Bone histomorphometry was performed on transiliac bone biopsy specimens. In order to perform dynamic measures of bone-formation activity, labeling with demeclocycline (15 to 20 mg/kg of body weight per day taken orally for two 2-day periods separated by a 10-day free interval) was performed prior to biopsy. Transiliac bone samples were collected 4 to 5 days after the labeling using a 5-mm Bordier trephine from a site located 2 cm below and behind the anterosuperior iliac spine. Biopsy preparation and histomorphometric analyses were performed as described previously.(13) Results for histomorphometric parameters were expressed as a percentage of the average results in subjects without metabolic bone disorders.(13) Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research.(14) The histomorphometric results of 19 patients had been included in a previous study on the material properties of OI bone.(15)

Statistical analysis

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.(16) Group differences in dichotomous variables were tested for significance using the chi-square test. Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Differences of Z-scores from 0 (for LS aBMD, height, weight) and data provided by the densitometer manufacturer.(10) The manufacturer data were based on the studies of Glastre and colleagues(11) and Southard and colleagues,(12) which were comprised of a total of 353 children and adolescents aged 1 to 19 years.

None of the patients included in this study had received bisphosphonate treatment prior to the densitometric measurement presented here. In patients who never received bisphosphonate treatment, the last available LS aBMD result was used for this analysis. In patients who were treated with bisphosphonates, the last LS aBMD before the start of bisphosphonate therapy was used.

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Results

A total of 192 patients (99 girls, 93 boys; age range 3 weeks to 16.9 years) were included in this study. The clinical diagnosis was OI type I in 78 patients, OI type III in 46 patients, and OI type IV in 68 patients. All 52 patients with a COL1A1 mutation leading to haploinsufficiency had a clinical diagnosis of OI type I (Table 1). Among the 140 patients with helical mutations, 26 (19%) had a clinical diagnosis of OI type I; the remainder were diagnosed with OI type III or IV.

Lumbar spine densitometry

In the patient population taken as a whole, LS aBMD Z-scores ranged from −7.8 to −0.6 and were not influenced by age (Fig. 1). LS aBMD Z-scores as well as Z-scores for height and weight were significantly below 0 for all three genotypic groups (p < .001 in each case), indicating that results were below the level expected for healthy age- and sex-matched subjects in each group. Compared with patients with helical mutations, patients

Table 1. Clinical characteristics in the study groups at the time of the bone densitometry analysis.

<table>
<thead>
<tr>
<th></th>
<th>Haploinsufficiency (n = 52)</th>
<th>Helical Mutation COL1A1 (n = 58)</th>
<th>Helical Mutation COL1A2 (n = 82)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (m/f)</td>
<td>22/30</td>
<td>29/29</td>
<td>40/42</td>
<td>0.58</td>
</tr>
<tr>
<td>OI type (I/III/IV)</td>
<td>52/0/0</td>
<td>10/21/27</td>
<td>16/25/41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.5 (4.8)</td>
<td>6.0 (5.0)</td>
<td>6.0 (4.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (z-score)</td>
<td>−1.3 (1.1)</td>
<td>−5.5 (3.1)**</td>
<td>−5.3 (3.3)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (z-score)</td>
<td>−1.0 (1.2)</td>
<td>−2.7 (1.9)**</td>
<td>−2.6 (1.7)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LS-aBMD (z score)</td>
<td>−3.4 (1.0)</td>
<td>−4.9 (1.5)**</td>
<td>−5.0 (1.3)**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Shown are n or mean (SD). The P value indicates the significance of the difference between groups (chi square or ANOVA, as appropriate). The results of the post-hoc analyses for comparisons between groups are shown in superscript:

**P < 0.001 for comparison to haploinsufficiency group. Differences between the two groups with helical mutations were not significant.
with COL1A1 haploinsufficiency on average were taller and heavier and had higher LS aBMD (Table 1). Differences in LS aBMD remained significant after adjustment for age, sex, and height Z-score. The adjusted means for LS aBMD were 4.0 (95% CI 3.6 to 4.3) for the haploinsufficiency group, 4.7 (95% CI 4.3 to 5.0) for the helical COL1A1 mutation group, and 4.7 (95% CI 4.5 to 5.0) for the helical COL1A2 mutation group. In the subgroup of patients with a clinical diagnosis of OI type I, LS aBMD Z-scores were similar in patients with haploinsufficiency mutations [3.4 (95% CI 3.1 to 3.7)] and in patients with helical glycine mutations [3.7 (95% CI 3.3 to 3.7)], p = .22.

In the whole patient population, average LS aBMD Z-score was higher by 0.6 (95% CI 0.2 to 1.0) in girls than in boys. This difference remained similar after adjustment for age and height Z-score. Similar trends with higher values in girls were found in each genotypic subgroup. The sex difference in LS aBMD Z-score was 0.5 (95% CI 0.1 to 1.1) in the haploinsufficiency group, 0.8 (95% CI 0.1 to 1.5) in patients with helical mutations in COL1A1, and 0.5 (95% CI 0.0 to 1.0) in patients with helical mutations in COL1A2.

In both the α1(I) and the α2(I) chain, serine substitutions were the most common type of mutation (Table 2). LS aBMD Z-scores were not significantly different between patients with serine substitutions and those with other substituting amino acids in either the α1(I) or the α2(I) chain. LS aBMD Z-scores did not correlate in a statistically significant manner with the location of the mutated residue in the α1(I) triple-helical domain whether analyzed in the entire group of patients with α1(I) mutations or in the subgroup with serine substitutions (Fig. 2). In patients with α2(I) mutations, a significant negative relationship was found between the position of serine substitutions and LS aBMD Z-score (Fig. 2). However, the partial correlation between the position of serine substitutions and LS aBMD Z-score was no longer significant after controlling for height Z-score (r = –0.16, p = .13). No significant relationship between the position of a glycine mutation and LS aBMD was found when this analysis was performed in the entire group of patients with α2(I) mutations.

![Figure 1](image1.png)

**Table 2. Relationship Between Specific Amino Acid Residue Substitutions for Glycine and LS aBMD Z-Scores**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>α1(I) Lumbar spine aBMD</th>
<th>α2(I) Lumbar spine aBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser</td>
<td>–4.7 (–7.4 to –2.1)</td>
<td>33 (–7.2 to –3.1)</td>
</tr>
<tr>
<td>Arg</td>
<td>–4.4 (–6.6 to –1.5)</td>
<td>13 (–6.7 to –3.5)</td>
</tr>
<tr>
<td>Asp</td>
<td>–5.6 (–7.1 to –4.0)</td>
<td>16 (–7.8 to –3.3)</td>
</tr>
<tr>
<td>Cys</td>
<td>–5.5 (–7.0 to –4.5)</td>
<td>7 (–7.3 to –3.2)</td>
</tr>
<tr>
<td>Ala</td>
<td>–5.4 (–7.5 to –5.1)</td>
<td>3 (–7.0 to –3.4)</td>
</tr>
<tr>
<td>Glu</td>
<td>–6.3</td>
<td>6 (–6.1 to –0.8)</td>
</tr>
<tr>
<td>Val</td>
<td>–5.4</td>
<td>7 (–6.1 to –2.9)</td>
</tr>
</tbody>
</table>

Results are given as median (range).

![Figure 2](image2.png)

**Figure 2.** Relationship between the positions of glycine substitutions in collagen type I α chains and LS aBMD Z-scores. The oblique drawn line represents the regression line between the position of Gly → Ser substitutions in α2(I) and LS aBMD Z-scores (r = –0.40, p = .02).
Iliac bone histomorphometry

Histomorphometric results were available for a subgroup of 96 patients [23 with haploinsufficiency mutations, 27 with helical α1(I) mutations, and 46 with helical α2(I) mutations]. As mentioned earlier, all patients with haploinsufficiency mutations were diagnosed with OI type I. The 73 patients with helical glycine mutations had the following phenotypic distribution: OI type I, n = 13; OI type III, n = 18; and OI type IV, n = 42. For each genotypic group, these results were expressed as a percentage of the average of the age-specific reference range for children and adolescents without bone disorder (Fig. 3).

Structural parameters (ie, core width, cortical width, and trabecular bone volume) were lower in all genotypic groups of OI patients than in healthy controls (Fig. 3). The comparison of structural parameters between OI groups showed that core width (outer bone size) and trabecular bone volume were similar between groups, but cortical width was significantly higher in the haploinsufficiency group (Fig. 4), whereas no significant differences were found between patients with α1(I) and those with α2(I) helical mutations.

Bone surface–based measures of bone formation and resorption (ie, osteoid surface, osteoblast surface, mineralizing surface, bone-formation rate, eroded surface, and number of osteoclasts) were significantly higher in all three groups of OI patients than in controls, with the exception of bone-formation rate, eroded surface, and number of osteoclasts in the haploinsufficiency group (Fig. 3). In contrast, osteoid thickness and mineral apposition rate were below the control average in all genotypic OI groups. Osteoid surface, osteoblast surface, bone-formation rate, eroded surface, and osteoclast number varied significantly with genotype, and for each parameter were lowest in the haploinsufficiency group. Bone-formation and -resorption parameters generally were similar between patients with α1(I) and α2(I) helical mutations, with the exception of eroded surface, which was significantly higher in patients with α1(I) helical mutations.

For helical mutations, there was no obvious relationship between the type of substituting amino acid or the position of the mutation and histomorphometric parameters. This is exemplified in Fig. 5 for a structural parameter, cortical width, and for a cellular parameter, osteoblast surface. In the subgroup of patients with a clinical diagnosis of OI type I, none of the histomorphometric parameters differed between patients with haploinsufficiency mutations (n = 23) and patients with helical glycine mutations (n = 13).

No significant sex differences were found for structural parameters either in the whole study population or in the genotypic subgroups. However, bone-formation rate expressed as a percentage of the age-specific reference mean values was significantly higher in boys than in girls both in the entire cohort (boys: median 153%, n = 46; girls: median 104%, n = 36; p = .02) and in the haploinsufficiency group (boys: median 123%, n = 10; girls: median 83%, n = 10; p = .05). The sex difference in bone-formation rate was not significant in the two helical mutation groups.

**Fig. 3.** Iliac bone histomorphometric results in patients with COL1A1 haploinsufficiency mutations (HI), as well as with COL1A1 and COL1A2 mutations leading to glycine substitutions in the helical domain of collagen type I. Results are expressed as a percentage of the average result in the age-specific reference range. The letters above the bars indicate the significance of the difference from 100% (ie, the average result in healthy controls): A: p < .05; B: p < .01; C: p < .001. The significance of the variation between genotype groups is indicated by the symbols above the bar: ns = not significant; “p < .05; “p < .01; “”p < .001. BFR/BS = bone-formation rate per bone surface; BV/TV = bone volume per tissue volume; CWi = core width; Ct.Wi = cortical width; ES/BS = eroded surface per bone surface; MAR = mineral apposition rate; MS/BS = mineralizing surface per bone surface; N.Oc/B.Pm = number of osteoclasts per bone perimeter; Ob.S/BS = osteoblast surface per bone surface; O.Th = osteoid thickness.

**Fig. 4.** Representative examples of iliac bone samples. (A) Haploinsufficiency mutation, 4-year-old boy with a c.1981C>T nucleotide change in COL1A1 that creates a stop codon (p.Gln661X). Core width 4.8 mm, cortical width 765 μm, bone volume per tissue volume 12.9%. (B) Helical glycine mutation, 9-year-old boy with a c.1090G>A nucleotide change in COL1A2 that creates an amino acid change (p.Gly364Ser). Core width 4.2 mm, cortical width 449 μm, bone volume per tissue volume 8.9%.
Discussion

In this study we observed that patients with COL1A1 mutations leading to haploinsufficiency have somewhat higher LS aBMD values than patients with helical mutations in \( \alpha_1(\text{I}) \) and \( \alpha_2(\text{I}) \). Patients with haploinsufficiency mutations had thicker iliac bone cortices and lower trabecular bone turnover than patients with helical mutations. Apart from these differences between patients with haploinsufficiency and helical mutations, no obvious genotype-phenotype relationships emerged. In patients with helical mutations, neither the type of \( \alpha \) chain affected, nor the type of amino acid substituting for glycine, nor the position of the mutation in the \( \alpha \) chain had a detectable influence on LS aBMD or histomorphometric results.

The lack of correlation between the type of helical mutation and LS aBMD may come as a surprise, given that previous studies have found that helical COL1A1 mutations lead to a more severe phenotype than helical COL1A2 mutations and that substitutions of branched or charged amino acids for glycine have more severe consequences than substitutions by other amino acids. However, these observations regarding genotype-phenotype correlations pertain mostly to the proportion of mutations of a given type that have a lethal outcome and therefore are not necessarily applicable to the present cohort, which included only patients with nonlethal mutations.

Most patients with helical mutations were more severely affected clinically than patients with haploinsufficiency mutations, as highlighted by an average height Z-score difference of 4 between the haploinsufficiency and helical mutation groups. In comparison, the LS aBMD Z-score differences between the haploinsufficiency and helical mutation groups were relatively modest: The average Z-score difference was about 0.7 after height adjustments. This relatively minor difference in LS aBMD is somewhat surprising considering that patients with helical mutations as a group have far more fragile bones than patients with haploinsufficiency mutations. However, our results are similar to those of Lund and colleagues, who in a study on 16 pediatric OI patients found that average total-body aBMD Z-scores differed by 0.6 between patients with “quantitative” (ie, haploinsufficiency) and “qualitative” mutations (presumably mostly helical glycine mutations) on protein analysis. This suggests that the more severe phenotype in patients with helical mutations is caused not only by differences in the amount of bone but also by other factors that remain to be elucidated.

In this study, 19% of patients with helical glycine mutations had a clinical diagnosis of OI type I. In comparison, the database of mutations associated with OI (Version 28, September 2009; www.le.ac.uk/ge/collagen/) reveals that approximately 10% (88 of 853) of the helical glycine mutations in \( \alpha_1(\text{I}) \) and \( \alpha_2(\text{I}) \) lead to a diagnosis of OI type I. The higher proportion of OI type I patients in our study may be due to the fact that the Sillence classification is inconsistently used among OI investigators. For example, some collagen sequencing laboratories give a diagnosis of OI type III/IV to all patients who have helical glycine mutations regardless of their phenotype. However, the Sillence classification is based exclusively on phenotypic criteria because it was devised before the molecular basis of OI was discovered. Consequently, we used only phenotypic criteria to diagnose OI types. This approach is also supported by our observation that the LS aBMD and histomorphometric results were similar in OI type I patients with haploinsufficiency and helical glycine mutations.
In our previous histomorphometric studies, we had observed that patients with OI type I had both more cortical and more trabecular bone than patients with OI types III and IV.(7) The present data showed that the genotypic groups differed mostly in the amount of cortical bone. Cortical thickness was about 50% higher in the haploinsufficiency group than in patients with helical mutations, whereas trabecular bone volume was similar between groups. This suggests that bone-mass differences between these genotypic groups are caused mainly by differences of bone modeling on periosteal and endocortical bone surfaces and less by differences in trabecular bone metabolism. The data also suggest that the classification of OI according to phenotypic types provides a better reflection of histologic disease severity than the grouping of patients according to mutation type.

The measurements of bone-formation and -resorption parameters on trabecular bone surfaces nevertheless provide some insights into the effect of collagen type I mutations on cellular activities. Mineral apposition rate was similarly low in all three genotypic groups, suggesting that individual osteoblasts produced bone matrix at a reduced rate regardless of the type of underlying mutation. This mirrors the results of our earlier studies, where patients were separated according to OI type.(7) Despite the sluggish bone formation at the individual cell level, bone-formation rate was increased in all genotypic groups. The reason for this apparent paradox is that osteoblast surface was two- to threefold above the average value of the reference range. Thus the individual OI osteoblast produces bone at a slower rate, but the larger than normal number of osteoblasts keeps the bone-formation rate high.

The fact that trabecular bone volume was low despite vigorous bone-formation activity shows that bone-resorption activity must also be high on the trabecular surfaces of OI bone. However, histomorphometry does not provide information on the rate of bone resorption, and therefore, this issue cannot be addressed directly. The percentage of trabecular surface with an eroded aspect was higher in patients with helical mutations than in patients with haploinsufficiency mutations. This is in accordance with the observation that bone-formation parameters also tended to be higher in the groups with helical mutations and especially in patients with α1(I) helical mutations, suggesting that bone turnover is elevated overall.

One of the new observations in this study was that girls with OI have slightly higher LS aBMD values than boys in the same genotypic group. This sex difference persisted after adjustment for height Z-score, suggesting that factors other than bone size are responsible for the higher LS aBMD values in girls. Our histomorphometric observations showed that bone-formation rate and thus bone turnover were lower in girls than in boys. Lower bone turnover is expected to lead to slightly higher bone mass because at any one time a smaller amount of bone will have been transiently removed by remodeling. It is thus possible that LS aBMD is higher in girls with OI because their bone turnover is less elevated than that of boys with the same disorder.

This study has several limitations. Even though the number of patients was an order of magnitude higher than in comparable previous studies, sample size was insufficient to address many important questions. For example, substitutions by many amino acid residues were too rare to gauge their phenotypic effect. The sample size also was insufficient to independently assess the effect of the position of a mutation and the type of substituting amino acid residue. In addition, this study provides information exclusively on the axial skeleton. Since bone density may vary considerably in different parts of the skeleton of OI patients, the present findings do not necessarily apply to the appendicular skeleton.(18)

In conclusion, compared with helical mutations in collagen type I α chains, COL1A1 mutations leading to haploinsufficiency are associated with higher LS aBMD values, thicker iliac bone cortices, and lower trabecular bone turnover. Among patients with OI owing to helical mutations, no relationship was found between densitometric or histomorphometric results and the type of amino acid substituting for glycine or the position of the mutation in the α chains. Thus patients with haploinsufficiency mutations had a milder skeletal phenotype than patients with mutations affecting glycine residues, but there was no clear genotype-phenotype correlation among patients with helical glycine mutations.

Disclosures

All the authors state that they have no conflicts of interest.

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