Collagen type Iα1 gene polymorphism in idiopathic osteoporosis in men


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Abstract

Objective. To analyse the distribution of polymorphism of the collagen type Iα1 gene (COL1A1) and its relationship with bone metabolism and bone turnover in men with idiopathic osteoporosis.

Methods. A total of 35 male patients with idiopathic osteoporosis, aged 50.4 ± 10.3 yr, and 60 healthy males (controls), aged 47 ± 17 yr, were included in the study. Serum osteocalcin, 25-hydroxyvitamin D and parathyroid hormone were determined in all patients. The COL1A1 Sp1 genotypes (SS, Ss, ss) were assessed by restriction enzyme digestion (BalI) of DNA amplified by the polymerase chain reaction.

Results. Patients with idiopathic osteoporosis had a higher frequency of the s allele than men in the control group (29 vs 11%, P = 0.003) and a higher frequency of the Ss genotype (patients, 48% SS, 46% Ss, 6% ss; controls, 80% SS, 18% Ss, 2% ss; P = 0.003). No significant differences between genotypes were observed in serum concentrations of osteocalcin, vitamin D or parathyroid hormone among either the patients or the controls.

Conclusion. This study suggests that, in men with idiopathic osteoporosis, there is a high prevalence of the s allele and the Ss genotype that is unrelated to other parameters of bone metabolism.

KEY WORDS: Bone mass, Osteopenia, Male, Genetic, Primary osteoporosis.

Evidence from family studies suggests that genetic factors have a major role in the determination of bone mass and in the development of osteoporosis [1, 2]. Although the genes responsible for these effects have not been defined completely, results of studies of polymorphisms of the vitamin D receptor (VDR) gene and the oestrogen receptor gene have been largely contradictory [3–8]. The reasons for these discrepancies remain to be determined, but the overall effect estimates suggest that VDR polymorphisms have a small effect on bone mass [8].

Type I collagen, a protein encoded by the COL1A1 and COL1A2 genes, is the major protein of the bone matrix. Genes encoding collagen type I may be important candidates for the genetic regulation of bone density, as mutations that affect the coding regions of these genes have been associated with osteogenesis imperfecta [9]. A polymorphism at the first base of a binding site for the transcription factor Sp1 in the first intron of the COL1A1 gene has recently been associated with low bone density and increased occurrence of osteoporotic fractures in women [10, 11]. Thus, heterozygotes at the polymorphic Sp1 site (Ss) had significantly lower bone mineral density than SS homozygotes, and bone mass was even lower in ss homozygotes. In addition, recent results have indicated that polymorphism of the COL1A1 gene may contribute to peak bone mass [12], and have also shown an increased risk of bone fractures in men in whom the s allele was over-represented [13].

While osteoporosis in women has been well documented, male osteoporosis has received much less attention, but it is increasingly recognized as a problem in clinical medicine. Although osteoporosis in men is frequently associated with an underlying secondary cause of bone loss, a large number of patients suffer from primary osteoporosis, also called idiopathic osteoporosis [14]. Few studies have focused on idiopathic osteoporosis in men, but it is likely that the pathogenesis is heterogeneous. The contribution of genetic factors to the pathogenesis of idiopathic male osteoporosis remains unknown.

Therefore, the aims of this study were to analyse the distribution of the COL1A1 polymorphism and its rela-
tionship with bone metabolism and bone turnover in men with idiopathic osteoporosis.

Patients and methods

The study was carried out on 35 Caucasian men with idiopathic osteoporosis aged 31–71 yr (50.4 ± 10.2 yr), who were attending the rheumatology service at our hospital and 60 healthy Caucasian men (control group) aged 21–86 yr (47 ± 17 yr). The patients were selected on the basis of the diagnosis of idiopathic osteoporosis. Osteoporosis was defined as the presence of one or more atraumatic vertebral crush fractures, or by densitometric criteria, i.e. a bone mineral density in the lumbar spine below two Z-score. Bone mineral density of the lumbar spine (L2–L4) was measured by dual X-ray absorptiometry using a bone mineral analyser (DPX-L; Lunar Radiation Corporation, Madison, WI, USA) and was expressed as g/cm². The in vitro and in vivo coefficients of variation were 0.5 and 0.8%, respectively. X-rays of the spine were obtained for all patients. A vertebral fracture was defined as a reduction of >20% in the anterior, middle or posterior height of the vertebral body.

An automated biochemical profile, complete blood count and determination of serum proteins and hormones, including testosterone, gonadotrophins, parathyroid hormone (PTH), urinary cortisol, 25-hydroxyvitamin D (25-OHD) and thyroid hormones, were performed in all patients in order to exclude an underlying secondary cause of osteoporosis. In addition, osteocalcin (BGP) was measured as a biochemical marker of bone formation in all patients. Serum BGP was measured by radioimmunoassay (Nichols Institute Diagnostics, San Juan de Capistrano, CA, USA). Serum 25-OHD was determined by a competitive protein-binding assay (Nichols Institute Diagnostics), and serum PTH was determined by an immunoradiometric assay (Nichols Institute Diagnostics).

Genomic DNA was extracted from samples of peripheral venous blood by standard proteinase K digestion, phenol–chloroform extraction and ethanol precipitation, and was used in the polymerase chain reaction (PCR). A mismatched primer was used, which introduced a restriction site for the enzyme Bal I in polymorphic alleles with the T substitution, as described previously [10]. After PCR, the reaction products were digested overnight with Bal I and analysed by agarose gel electrophoresis. This assay discriminates two alleles, S and s, and three polymorphic genotypes, SS, Ss and ss.

Group mean values of patients and controls were compared by the use of the Mann–Whitney U-test. The Kruskal–Wallis test was used to compare the BGP, PTH and 25-OHD concentrations of the three genotype groups of patients and controls. A P value of less than 0.05 was considered significant. Results are expressed as mean ± s.d. The χ² exact test, with correction for continuity, was used to compare allele and genotype frequencies between cases and controls. Hardy–Weinberg calculations were performed to test for disequilibrium.

Results

Table 1 shows the distribution of COL1A1 genotypes in the patients and controls. The allelic frequencies (89% for S and 11% for s) and the distribution of genotypes (80% SS, 18% Ss, 2% ss) in the control group were in Hardy–Weinberg equilibrium and were similar to those reported in previous studies [10–12]. Patients with idiopathic osteoporosis, however, showed higher allelic frequencies for the s allele (71% for S and 29% for s) and for the Ss genotype (48% SS, 46% Ss, 6% ss). The allelic and genotype distributions showed significant differences between osteoporotic patients and controls (Table 1). Because of the low frequency of cases and controls with the ss genotype, data for SS and ss individuals were combined.

As whole groups, controls and patients did not differ in BGP (controls 5.7 ± 2.3 ng/ml; patients 5.7 ± 0.9 ng/ml; P > 0.05), 25-OHD (controls 22.1 ± 10.6 ng/ml; patients 19.4 ± 8.2 ng/ml; P > 0.05) or PTH (controls 33.9 ± 13 pg/ml; patients, 32.1 ± 13.8 pg/ml; P > 0.05). Similarly, no significant differences between genotypes were observed in the serum levels of BGP, PTH or 25-OHD in the controls and in the osteoporotic patients (Table 2).

In patients with idiopathic osteoporosis, no significant differences in lumbar bone mineral density between genotypes were observed (SS patients 0.859 ± 0.09 g/cm², Ss + ss patients 0.877 ± 0.09 g/cm²; P > 0.05) or in lumbar T score (SS patients −3.12 ± 0.7, Ss + ss patients −3.04 ± 0.7; P > 0.05). Fourteen patients (40%) had vertebral fractures; nine of them were in the SS group and the remaining six were in the Ss + ss group (P > 0.05). The low number of patients with vertebral fractures does not permit us to draw a firm conclusion from this result.

Table 1. Genotype and allele frequencies in patients and controls

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<thead>
<tr>
<th>Allele frequency</th>
<th>Genotype frequency</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
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<tr>
<td>Controls (n = 60)</td>
<td>107 (89%)</td>
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<tr>
<td>Patients (n = 35)</td>
<td>50 (71%)</td>
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<td>P = 0.003</td>
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Table 2. Parameters of bone metabolism in function of genotype (mean ± s.d.)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>Ss + ss</td>
</tr>
<tr>
<td>BGP (ng/ml)</td>
<td>5.6 ± 2.2</td>
<td>6.1 ± 2.8</td>
</tr>
<tr>
<td>25-OHD (ng/ml)</td>
<td>23 ± 11</td>
<td>20 ± 10</td>
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<tr>
<td>PTH (pg/ml)</td>
<td>35 ± 15</td>
<td>29 ± 10</td>
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There were no significant differences between genotype groups (Kruskal–Wallis test).
Discussion

This study shows high prevalences, in men with idiopathic osteoporosis, of the s allele and the Ss genotype at the COL1A1 locus which is unrelated to other parameters of bone metabolism. The genotype distribution in the control group was similar to that reported in previous studies in Caucasian populations [10–12].

Data on genetic studies in male osteoporosis are scarce, especially with regard to male idiopathic osteoporosis. Thus, only one previous study has been performed in men with idiopathic osteoporosis [15]. In this study the VDR gene polymorphisms were analysed in a group of 20 patients, and no significant over-representation of any VDR genotype was found. Langdahl et al. [13], in a recent series, investigated the relationship between the COL1A1 Sp1 polymorphism and osteoporosis in a case–control study which included men and women with osteoporotic vertebral fractures. The cause of the osteoporosis in these patients was not commented on in the paper. The authors observed over-representation of the ss genotype in both sexes and, as in our study, male patients with osteoporosis showed over-representation of the s allele, whereas the heterozygotes did not show an increased risk of osteoporosis. These discrepancies were considered to be related to the small sample size in both series.

Recent studies have observed an association between COL1A1 polymorphism and low bone mass in women from various European populations [10–12]. Moreover, osteoporotic women with vertebral fractures have also shown over-representation of the s allele, suggesting that the COL1A1 polymorphism could be of clinical value as a predictor of osteoporotic vertebral fractures. In addition, and in agreement with the present results, we have recently shown over-representation of the s allele in premenopausal women with primary osteoporosis [16]. Willing et al. [7] in a previous report did not find any association between bone mass and the polymorphism of COL1A1. Although these authors studied other polymorphic sites of COL1A1 with dissimilar results, these findings may also be a reflection of the complex genetics of a trait such as bone density, for which studies sometimes show discordant results.

We found no association between PTH, vitamin D or BGP levels and COL1A1 polymorphism, which is consistent with most of the previous reports showing no association of genotypes with bone markers [13]. Nevertheless, in a recent study Keen et al. [17] showed increased urinary levels of pyridinoline in patients with the s allele, suggesting an increase in bone resorption in these patients. Interestingly, Garnero et al. [12] indicated a decrease in serum levels of C-terminal propeptide (PICP) of type I procollagen in patients with the ss genotype, suggesting a decreased rate of synthesis of type I collagen, but other markers of bone turnover, such as the N-terminal propeptide of procollagen type I (PINP) and the urinary carboxy- and amino-terminal telopeptides (CTX and NTX) of type I collagen, were not related to collagen polymorphisms. Moreover, Langdahl et al. [13], who also studied PICP in relation to genotypes of collagen, did not confirm these results. All these findings are consistent with the hypothesis that the COL1A1 genotype predisposes to osteoporosis by way of a direct effect on bone mass, through an action on the bone collagen matrix. Thus, Uitterlinden et al. [11] indicated that COL1A1 polymorphism may act as a marker of differences in bone quality, such as bone structure or matrix composition, as the relative risk of fracture found in their study was greater than that predicted by the differences between genotypes in bone density.

The cellular and molecular events that underlie the association between COL1A1 alleles and bone mass remain unclear. It has been indicated that the s allele sequences bind the Sp1 protein with greater affinity than those of the S allele, resulting in three times as many transcripts. It is possible that the presence of the s allele causes variation in the relative quantities of COL1A1 and COL1A2 messenger RNAs, producing an abnormal collagen similar to that described in osteogenesis imperfecta [11]. Since approximately 80% of the protein content of bone is type I collagen, it is to be expected that a defect in this protein will produce bone fragility.

In conclusion, the results of this study suggest an association between COL1A1 genotype and male idiopathic osteoporosis. However, the low sample size of the cases studied in this report could mean that the apparent association detected is a false as opposed to a true positive. This indicates the need to study additional cohorts to confirm our findings.

Acknowledgements

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References