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Ethnic Difference in Contribution of Sp1 Site Variation of *COLIA1* Gene in Genetic Predisposition to Osteoporosis

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Abstract. Osteoporosis, a condition characterized by low bone mineral density (BMD) leading to bone fragility [1], is a major public health concern in Japan as well as in other countries. Although genetic predisposition seems to be a factor in the pathogenesis of osteoporosis [2–4], the precise cohort of genes that may be involved is not well defined. The *COLIA1* and *COLIA2* genes encode polypeptide constituents of collagen type Iα1 and Iα2, respectively. Both are important candidates as genetic regulators of BMD, since mutations in either gene result in osteogenesis imperfecta, a disorder characterized by severe osteoporosis [5]. Some patients with adult osteoporosis also carry mutations in *COLIA1* or *COLIA2* genes [6].

Three independent studies performed in Caucasian populations [7–9] have demonstrated a genetic association between allelic variants of *COLIA1* and reduced BMD. In all three studies, a guanine-to-thymidine change (2046T allele) at the recognition site for transcription factor Sp1 in the first intron of the *COLIA1* gene (base +2046 of intron 1) showed a significant association with reduced bone density and increased occurrence of osteoporotic fracture. Moreover, Uitterlinden et al. [9] showed that Sp1 protein binds to the sequence containing 2046T with greater affinity than to 2046G sequence, and that this action alters expression of the *COLIA1* gene. These observations suggested that the nature of the Sp1-binding sequence of *COLIA1* might play a critical role in the pathogenesis of osteoporosis.

To examine whether genetic variation of the *COLIA1* gene is associated with osteoporosis in the Japanese population, we studied the relationship between *COLIA1* genotype and BMD in 202 postmenopausal Japanese women with osteoporosis (mean age 72.4 ± 5.6 years, adjusted-BMD at radius 0.28 ± 0.03 g/cm^2) and 202 control women (mean age 68.7 ± 6.36, adjusted-BMD at radius 0.39 ± 0.05 g/cm^2). DNA samples were obtained from all participants with informed consent. Osteoporosis was diagnosed according to criteria of the Japanese society for bone and mineral research. *COLIA1* genotypes were determined by PCR technology, using a mismatched primer that introduces a cleavage site for endonuclease *MscI* in alleles that carry a 2046T substitution [7]. In this procedure, reaction products digested with *MscI* yield a smaller fragment for the 2046T allele (Fig. 1).

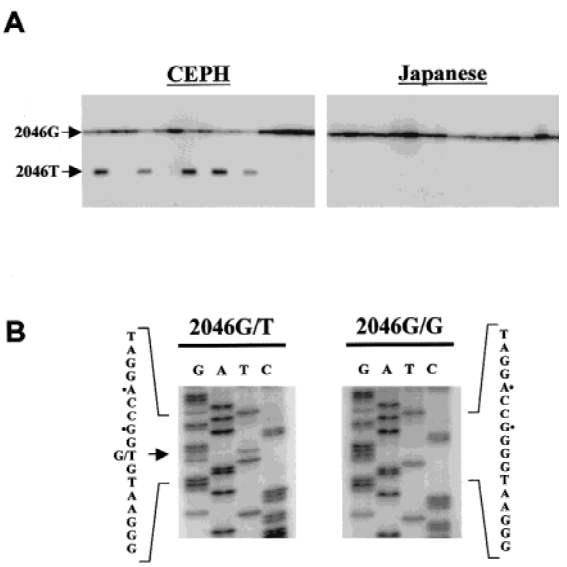

Fig. 1. (A) Representative mismatched PCR-RFLP genotyping showing variation in the Sp1-binding site of *COLIA1* in 16 Caucasians but not in 16 Japanese. PCR products containing a substitution of T for G at nucleotide 2046 contained a restriction site for *MscI*; digestion produced a smaller allele in heterozygotes. **(B)** Sequencing experiment showing heterozygosity at nucleotide 2046 (intron I) of *COLIA1* in a CEPH DNA sample and homozygosity for 2046G in a Japanese sample. The replaced nucleotide by mismatched primer (●). The sequencing results were consistent throughout the test populations.

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Table 1. Allele frequency of G-to-T polymorphism in the first intron of *COLIA1* gene

Population	Number	Allele frequency		Reference
		2046G	2046T	
United Kingdom	299	0.80	0.20	[7]
Netherlands	1778	0.82	0.18	[9]
France	220	0.78	0.22	[8]
Japan	404	1.00	0.00	

The allelic frequency of 2046T previously reported in Caucasians was 0.20–0.22 [7–9]. However, among our 202 Japanese patients and 202 controls, none carried the 2046T allele at the Sp1 binding site (Table 1). When we genotyped 40 unrelated Caucasian subjects from CEPH kindreds, we detected 13 2046G/T heterozygotes (allele frequency of 2046T, 0.16). Sequence analysis of PCR products from each allele confirmed these findings (data not shown).

Individuals belonging to different racial groups often show differences in susceptibility to common polygenic diseases. That osteoporosis is less common among blacks than Caucasians and Mongoloids [10] suggests that genetic background is a major factor affecting susceptibility to osteoporosis. Variants of other candidate genes, such as vitamin D receptor, have been associated with reduced bone density in some studies but not in others [11, 12]. Ethnic differences might explain those disparities. For example, Tokita et al. [13] reported that genetic variation of the vitamin D receptor gene significantly affected BMD in Japanese women, even though the frequencies of haplotypes and genotypes of this gene were quite different from those observed in Caucasians. *COLIA1* variations are likely to contribute to reduced BMD in Caucasians because the sample size in one of the reported studies was extremely large [9] and because those findings were consistent with two other independent studies [7, 8]. Nevertheless, the 2046T allele clearly does not predispose to osteoporosis in Japanese women, since we have shown that it is not present in the Japanese population.

The results reported here emphasize the importance of considering racial background in assessing the etiological significance of candidate genes in osteoporosis.

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