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ORIGINAL ARTICLE

Association between osteoprotegerin gene polymorphism and bone mineral density in patients with adolescent idiopathic scoliosis

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Abstract Generalized low bone mass and osteopenia have been reported in the axial and peripheral skeleton of adolescent idiopathic scoliosis (AIS) patients. Recently, many studies have shown that gene polymorphisms are related to osteoporosis. However, no studies have linked the association between gene polymorphisms and bone mass of AIS. Therefore, this study examined the association between the bone mass and RANKL, RANK, and OPG gene polymorphisms in 198 girls diagnosed with AIS. OPG 163 A → G, 209 G → A, 245 T → G, and 1181 G → C polymorphisms; RANK 421 C → T and 575 C → T polymorphisms; and RANKL rs12721445 and rs2277438 polymorphisms, as well as the bone mineral density at the lumbar spine (LSBMD) and femoral neck (FNBMD) were analyzed. The 163 A → G, 209 G → A, and 245 T → G polymorphisms in the OPG gene were in complete linkage. No RANK 421 C → T and 575 C → T polymorphisms or RANKL rs12711445 polymorphism were observed. There was a significant association between the OPG gene 1181 G → C polymorphism and LSBMD. LSBMD in AIS with the CC genotype was found to be significantly higher than in AIS with the GC ($P < 0.05$) or GG ($P < 0.01$) genotype. However, there was no significant association between LSBMD or FNBMD and the OPG gene 245 T → G polymorphism or the RANKL rs2277438 polymorphism. These results suggest that the OPG gene 1181 G → C polymorphism is associated with LSBMD in girls with AIS.

Keywords Adolescent idiopathic scoliosis · Bone mineral density · OPG · RANKL · Polymorphism

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Introduction

Adolescent idiopathic scoliosis (AIS) is a complex three-dimensional deformity of the spine occurring mostly in girls aged between 10 and 14 years old. The etiology and pathogenesis of idiopathic scoliosis is unclear despite the large number of studies reported. The cause of scoliosis is believed to be multifactorial due to the association between the development of scoliosis and growth, hormonal secretion, gravity, etc. [1, 13, 14, 16, 20, 22, 23, 25, 27, 30]. However, none of these parameters have been demonstrated to play a causative role in the development of AIS.

Burner et al. [6] first reported an association of osteopenia with idiopathic scoliosis using the Singh index. Generalized low bone mass and osteopenia in both the axial and peripheral skeleton in AIS have been reported in the literature [7, 9, 12, 26, 28]. Abnormal histomorphometric bone cell activity has been observed in AIS bone biopsies [10]. In addition, the low bone mass in AIS patients is likely to persist through to adulthood [8]. There is increasing concern that adolescents with idiopathic scoliosis might have a lower peak bone mass, thereby increasing the risk of developing osteoporosis and related complications in later life [8, 9]. However, the precise mechanism and causes of bone loss in AIS have not been identified.

Osteoporosis is characterized by a decrease in skeletal mass caused an imbalance between bone resorption and bone formation. Recently it was reported that osteoclast formation and activity are regulated by three members of the tumor necrosis factor and tumor necrosis factor receptor superfamily: receptor activator of nuclear factor-κB (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG). RANKL is a potent stimulator of bone resorption by binding RANK in the cell membrane of osteoclasts. In contrast, OPG is a soluble decoy receptor for RANKL, which interferes with RANKL/RANK binding and inhibits the maturation and activation of osteoclasts and their precursors. The balance between RANKL/RANK and OPG plays a key role in regulating the bone remodeling events in diseases, such as osteoporosis, glucocorticoid-induced osteoporosis, chronic inflammatory arthritis, hypogonadism, estrogen deficiency, bone marrow transplantation, and the osteolytic bony metastasis of malignancies [5, 15, 17]. Therefore, the genes of any component of the OPG–RANK–RANKL system might be candidate loci for the osteoporosis gene.

Several investigators have evaluated the association between OPG gene polymorphisms and BMD in postmenopausal women [2, 3, 11, 19, 21, 24, 29, 32, 33]. In addition, some studies examined the relationships between the RANK and RANKL gene polymorphisms and BMD in postmenopausal women [11, 18, 19]. However, there are no reports linking the association between the bone mass of AIS patients and RANKL, RANK, and OPG gene polymorphisms. This study examined the association between bone mass in girls with AIS and RANKL, RANK, and OPG gene polymorphisms.

Materials and methods

One hundred ninety-eight girls (11 and 13 years of age) newly diagnosed with AIS were enrolled at the authors' institution. Patients receiving any form of prior treatment for scoliosis were excluded. Subjects with a history of congenital deformities, neuromuscular disease, endocrine disease, skeletal dysplasia, connective tissue abnormalities or mental retardation were excluded. All subjects and their parents provided informed consent before the examination and measurements. The study was approved by the Clinical Research Ethics Committee of the university and hospital.

Evaluation of severity of scoliosis

Normal standing whole spine antero-posterior radiographs were taken for each AIS patient at the first presentation. A standard technique was used to measure the Cobb's angle. If more than one curve was found, the most severe curve was selected for the measurement. Curves <10° were excluded.

Anthropometric measurement

Anthropometric measurements included the body height and body weight. A normal standing whole spine antero-posterior radiograph was taken for each AIS patient. A standard technique was used to measure the Cobb's angle. For the AIS patients, the corrected height was derived from Bjure's formula (Log $y = 0.011x - 0.177$, where y is the loss of trunk height (cm) due to the deformed spine and x is the greatest Cobb angle of the primary curve) [8]. The body mass index (BMI) was determined by dividing the weight (kg) by the square of the uncorrected height (m^2).

Dual energy X-ray absorptiometry

The lumbar spinal bone mineral density (LSBMD) and femoral neck BMD (FNBMD) of the non-dominant proximal femur were measured by dual-energy X-ray absorptiometry (DEXA) (XR-36; Norland Corp., Fort Atkinson, Wisc., USA). The LSBMD was measured in L1–L4 in the anterior–posterior view. The scoliotic curvature of the spine in the AIS patients might present difficulties in measuring the spinal BMD reliably. In order to minimize this problem, the level of rotation in patients with AIS was determined by pre-scanning the spine and measuring the LSBMD in the neutral position [26].

Determination of the OPG 163 A → G, 209 G → A, 245 T → G, and 1181 G → C polymorphisms

The genomic DNA was extracted from the peripheral blood leukocytes using a QIAamp DNA blood kit (Qiagen GmbH, Hilden, Germany). The genomic DNA containing the 209 G → A polymorphic portion in the OPG promoter was amplified by PCR, as described [3]. The 163 A → G, 245 T → G and 1181 G → C polymorphisms were analyzed by amplifying the specific DNA fragments by PCR using the oligonucleotide primers and PCR reaction steps described elsewhere [21]. The PCR products were digested with *Ase*I, *Taq*I, *Hin*fI, and *Sml*I restriction endonuclease to detect the 163 A → G, 209 G → A, 245 T → G, and 1181 G → C polymorphisms, respectively, and electrophoresed through agarose gels containing ethidium bromide. The gels were visualized on a transilluminator under ultraviolet light and photographed.

Determination of the RANK 421 C → T and 575 C → T polymorphisms

The 421 C → T polymorphism with exon 4 and the 575 C → T polymorphism within exon 6 of the RANK gene were analyzed by amplifying the specific genomic DNA fragments containing these polymorphic portions by PCR

using the oligonucleotide primers described elsewhere [31]. The PCR products were digested with *Rsa*I and *Hha*I restriction endonuclease, respectively, and electrophoresed through agarose gels containing ethidium bromide. The gels were visualized on a transilluminator under ultraviolet light and photographed.

Determination of the RANKL rs12721445 and rs2277438 polymorphisms

The polymorphic regions of the RANKL gene were amplified by polymerase chain reaction (PCR) with the specific forward primers (AAGATCTCCAACATGACTT TTAGCAA for rs1272445 and ACATGTGATTTCCT TGGTGCC for rs2277438) and with the specific reverse primers (ATCCAGGAAATACATAACACTCCAAA for rs1272445 and TGGAGTCTCAAAATTCTATAAATG CAG for rs2277438). The PCR products were electrophoresed through 1.5% agarose gel to confirm the reaction and purified directly from PCR using a PCR clean kit (Qiagen GmbH). Their sequences were determined by cycle sequencing using an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) on an automated DNA sequencer (ABI PRISM 310, Perkin Elmer Applied Biosystems, Foster City, CA).

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software for Windows (SPSS, Chicago, IL, USA). The data are expressed as the mean ± standard deviation. The groups were compared using a *t* test and ANOVA, wherever appropriate. A $P < 0.05$ was considered significant.

Results

Single polymorphisms

The 163 A → G, 209 G → A, and 245 T → G polymorphisms in the OPG gene were in complete linkage. The distributions of the OPG 245 T → G and 1181 G → C polymorphisms and RANKL rs2277438 polymorphism were follows: TT 81.7, TG 18.3, GG 55.5, GC 38.0, CC 6.5, AA 40.8, AG 46.2, and GG 13.0%. These allele frequencies followed Hardy–Weinberg equilibrium. No RANK 421 C → T and 575 C → T polymorphisms and the RANKL rs12711445 polymorphism were observed.

There was a significant association between the OPG gene 1181 G → C polymorphism and LSBMD (Table 1). The LSBMD in AIS patients with the CC genotype was significantly higher than in the AIS patients with the GC ($P < 0.05$) or GG ($P < 0.01$) genotypes. However, there was no significant association between LSBMD or FNBMD and the OPG gene 245 T → G polymorphism or RANKL rs2277438 polymorphism.

There were no statistically significant differences in age, Cobb's angle, BMI, and cBMI in the patients with the OPG 245 T → G, OPG 1181 G → C, and RANKL rs2277438 genotypes.

Combined polymorphisms

Three different haplotype alleles (TG, TC, GG) were identified by haplotype analysis of the 245 T → G and 1181 G → C polymorphisms of OPG in combination. Five different genotypes, TGTG (48.0%), TGTC (25.3%), TGGG (10.1%), TCGG (8.6%), and TCTC (8.0%), were

Table 1 Clinical characteristics and bone mineral density in girls diagnosed with AIS in relation to OPG and RANKL polymorphisms

	Age	Cobb's angle	BMI	cBMI	LSBMD	FNBMD
OPG 245T → G						
TT ($n = 160$)	12.5 ± 0.8	25 ± 12	17.7 ± 1.6	17.4 ± 1.6	0.717 ± 0.052	0.704 ± 0.045
TG ($n = 38$)	12.6 ± 0.8	26 ± 11	18.2 ± 1.6	17.9 ± 1.6	0.716 ± 0.043	0.707 ± 0.042
P value	0.3674	0.4592	0.0870	0.0917	0.8905	0.6274
OPG 1181G → C						
GG ($n = 114$)	12.4 ± 0.8	24 ± 9	17.9 ± 1.6	17.6 ± 1.5	0.711 ± 0.042	0.704 ± 0.042
GC ($n = 67$)	12.7 ± 0.8	27 ± 14	17.8 ± 1.7	17.5 ± 1.7	0.712 ± 0.050	0.705 ± 0.044
CC ($n = 17$)	12.7 ± 0.7	26 ± 10	17.1 ± 1.9	16.8 ± 1.9	0.772 ± 0.072	0.709 ± 0.057
P value	0.0735	0.1150	0.1497	0.1699	0.0010	0.6941
RANKL						
AA ($n = 85$)	12.5 ± 0.8	25 ± 12	18.0 ± 1.7	17.6 ± 1.8	0.718 ± 0.050	0.703 ± 0.045
AG ($n = 83$)	12.6 ± 0.8	26 ± 12	17.7 ± 1.5	17.4 ± 1.5	0.718 ± 0.052	0.708 ± 0.044
GG ($n = 30$)	12.3 ± 0.7	22 ± 7	17.8 ± 1.7	17.5 ± 1.7	0.710 ± 0.047	0.700 ± 0.044
P value	0.2712	0.2627	0.5586	0.5861	0.7183	0.0602

cBMI Corrected BMI

identified when these haplotypes were combined. However, these haplotypes or genotypes were not associated with LSBMD or FNBMD.

When the OPG 1181 G → C and RANKL rs2277438 polymorphisms were combined, there were five genotypes with a frequency >5%: GGAA (27.8%), GGAG (17.7%), GCAG (19.2%), GGGG (11.6%), and GCAA (11.1%). These five genotypes also had no significant association with BMD.

Discussion

Generalized low bone mass and osteopenia in both the axial and peripheral skeleton of AIS patients have been reported [7, 9, 12, 26, 28]. However, the precise mechanism of bone loss in AIS patients is unclear. Recently, many studies reported that gene polymorphisms were related to osteoporosis. However, there are no reports linking the association between gene polymorphisms and bone mass of AIS.

In order to identify the genes involved in the regulation of bone mass in Korean AIS patients, who represent an ethnically homogenous population, this study examined the OPG gene 163 A → G, 209 G → A, 245 T → G, and 1181 G → C polymorphisms; the RANK gene 421 C → T and 575 C → T polymorphisms; and the RANKL rs12721445 and rs2277438 polymorphisms. The OPG gene 1181 G → C polymorphism is associated with LSBMD in AIS patients.

The prevalence of the three OPG 1181 G → C genotypes in these subjects was GG 57.6, GC 33.8, and CC 8.6%. AIS patients with the C allele had a significantly higher LSBMD but not FNBMD. These findings are in agreement with those reported by Kim et al. [19] and Choi et al. [11], who measured the BMD in postmenopausal Korean women. In addition, the polymorphism was associated with a preserved bone mass at the lumbar spine in 1181 CC homozygosity, whereas 1181 GG homozygosity and 1181 GC heterozygosity were more common in patients with osteoporosis [21]. However, several other authors reported no significant relationship between the OPG 1181 G → C genotype and BMD [24, 29]. This suggests that the prevalence of the genotype of the OPG 1181 G → C polymorphism and their genotypic effects on bone mass might differ between different ethnic groups or different countries with the same ethnicity. However, the mechanism for how OPG 11811 G → C polymorphism affects BMD is unknown, even though changing the third amino acid of OPG from a basic lysine to asparagine might affect the OPG secretory kinetics [4].

This study confirmed the complete linkage of polymorphisms at position 163 A → G, 209 G → A, and 245 T → G. Therefore, the 245 T → G polymorphism was chosen as a representative for analysis. Although a recent study reported no significant association between OPG polymorphisms and osteoporosis in a Japanese population [24], a study of a cohort of postmenopausal Slovenian women with osteoporosis reported that polymorphisms 209 G → A and 245 T → G in the promoter region of OPG were negatively correlated with the BMD at the lumbar spine but not at the femoral neck [3]. Regarding the 163 A → G polymorphism, this finding is accordance with the results reported by Hsu et al. [18] in a Chinese population.

RANK 421 C → T and 575 C → T polymorphisms were not observed in this study, whereas there was a high frequency of the RANK 421 C allele and 575 T allele in Caucasians [31]. No RANKL rs12711445 polymorphism located in exon 6 was observed. On the other hand, the RANKL rs2277438 polymorphism is located in intron 2 with an A allele frequency of 0.64. However, the RANKL rs2277438 polymorphism was not found to be associated with BMD in Korean AIS patients.

A study using haplotype analysis is believed as an effective tool for determining the genetic contributions of common diseases. However, haplotype analysis on the OPG 245 T → G and 1181 G → C polymorphisms in combination showed no significant association with the BMD. This suggests that the OPG 1181 G → C polymorphism has no significant effects on bone mass. In this study, the combined OPG 1181 G → C and RANKL rs2277438 genotype were also not significantly associated with the BMD. This indicates that a gene-to-gene interaction between the polymorphisms in the OPG and RANKL genes is not significant to modulate the BMD.

There were some limitations of this study. The number of samples tested was relatively small, which diminishes its statistical power and the possibility of detecting correlations. Further studies with a larger patient population are recommended. The association with other factors, such as the markers of bone metabolism, bone quality, and also other candidate genes, should be tested.

In summary, this study examined the association between the BMD and RANKL, RANK, and OPG gene polymorphisms in Korean girls diagnosed with AIS. The OPG gene 1181 G → C polymorphism was found to influence LSBMD but the definite mechanisms for the low bone mass in AIS is unknown. Therefore, further study with a larger number of subjects will be needed.

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