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Original article

Osteoprotegerin genetic polymorphisms and age of symptom onset in ankylosing spondylitis

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Abstract

Objectives. Osteoporosis is one of the recognized features of AS. It is known that RANK ligand (RANKL), which binds to RANK, can cause the activation of bone resorption. Osteoprotegerin (OPG) also competes with RANK by binding to RANKL and inhibiting bone absorption. Therefore, we designed a case–control study to evaluate the association between occurrence and clinical features of AS and *RANK*, *RANKL* and *OPG* genetic polymorphisms.

Methods. A total of 330 AS patients and 330 age- and gender-matched controls were recruited. PCR-restriction fragment length polymorphism was applied to identify *RANK* C575T, *RANKL* C-290T and *OPG* G1181C genotypes.

Results. *OPG* GG genotype carriers had an elevated risk of AS compared with those with the GC and CC genotypes (matched odds ratio 1.74; 95% CI 1.26, 2.40). Age of symptom onset and frequency of peripheral arthritis significantly differed among AS patients by *OPG* G1181C genotypes. *HLA-B27*$^+$ patients with the *OPG* C allele had the earliest age of symptom onset [mean (S.D.) 26.6 (9.6) years], followed by *HLA-B27*$^+$ patients with the *OPG* G allele [32.6 (12.2) years], *HLA-B27*$^-$ patients with the *OPG* G allele [38.1 (13.6) years] and *HLA-B27*$^-$ patients with the *OPG* C allele [38.6 (9.8) years].

Conclusion. *OPG* G1181C polymorphism may be associated with AS development and clinical manifestations.

Key words: Age of symptom onset, Ankylosing spondylitis, Osteoprotegerin, Polymorphism.

Introduction

AS is a chronic inflammatory disorder of the lumbar spine and SI joint that can also affect the peripheral joints [1]. Males are affected more frequently than females [2], and AS development is not completely understood. AS is strongly associated with the *HLA-B27* gene [3], but *HLA-B27* accounts only for 16% of the genetic variability in AS [4].

Osteoporosis and fractures of the vertebral body are recognized features of AS [5, 6]. Osteoporosis is caused by bone resorption through osteoclasts rather than bone formation through osteoblasts [7]. Bone modelling is regulated through the interaction of osteoclasts and osteoblasts through the RANK ligand (RANKL), RANK and osteoprotegerin (OPG) pathways [8]. RANKL produced by osteoblasts interacts with RANK on the surface of osteoclast precursors, resulting in differentiation and maturation of osteoclasts and further activation of bone resorption [9, 10]. OPG, a novel member of the TNF receptor superfamily [11], binds with RANKL to regulate bone resorption by inhibiting osteoclast differentiation and activation and inducing osteoclast apoptosis [12–14]. OPG-deficient mice develop severe osteoporosis [15], while overexpression of OPG in transgenic mice produces profound osteopetrosis [11].

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Human *RANKL* is located on chromosome 13q14 [16]. Sequence variations in the *RANKL* promoter region may alter the binding of various transcription factors [17]. The *RANK* C575T genotype (rs1805034) is the only known non-synonymous polymorphism at exon 6 (on chromosome 18q22.1) resulting in the amino acid variation from valine to alanine and as such may affect the protein product [18, 19]. Binding of RANKL with RANK is critical in osteoclast activation and differentiation, so the *RANKL* C-290T and *RANK* C575T polymorphisms are potential markers of AS. Human *OPG* gene is located on chromosome 8q24 [20]. The G1181C genotype in the first exon of the *OPG* gene changes the third amino acid of the signal peptide from lysine (Lys) to asparagine (Asn) [21]. This, the only known non-synonymous polymorphism in the signal peptide, affects cellular secretion of OPG [22]. OPG competes with RANK to inhibit activation of osteoclasts and plays a protective role in bone resorption. Therefore, OPG may be important in AS pathogenesis.

In the present study, we examined the association of AS occurrence with *RANK* C575T, *RANKL* C-290T and *OPG* G1181C genetic polymorphisms. Disease severity and complications may be largely determined by genetic factors in AS patients [23]. Subjects with earlier onset of symptoms also have more complications and more severe disease [24]. However, the association of genetic factors and age of symptom onset in AS has not been evaluated. AS complications may also be associated with genetic factors [25, 26]. Therefore, we also evaluated the relationship of clinical features with genetic polymorphisms of *RANK*, *RANKL* and *OPG*.

Materials and methods

Patients and controls

The study conformed to the Declaration of Helsinki and was approved by the relevant ethics committee of Chung Shan Medical University Hospital, Taichung, Taiwan. Patients who met the selection criteria were solicited sequentially from the hospital outpatient arthritis clinic from May 2004 to September 2008 to participate in the study. Informed consent was obtained before any data were collected from respondents. Selection criteria were: (i) age >18 years; (ii) AS diagnosis by the modified New York criteria [27]; (iii) spoke fluent Chinese; and (iv) unimpaired cognitive performance (e.g. no dementia). Sacroiliitis was confirmed by a qualified radiologist and AS by a qualified rheumatologist. In total, 330 unrelated AS patients were recruited. A detailed clinical history was taken by a physician at enrolment, including age of symptom onset, family history of AS and extraspinal manifestations. Age of AS symptom onset was defined at the time when the first symptom, whether it was axial symptom, peripheral arthritis, uveitis or enthesitis, had developed. Peripheral arthritis was defined as the presence of at least one swollen joint. General health questionnaires were completed at the time of sample collection to find individuals to serve as controls. Potential controls were randomly selected from sequential patients with no significant medical histories or abnormal laboratory results. These individuals were admitted to the same medical centre for routine physical exams and were from the same geographic areas as cases. A 1 : 1 ratio of case to control subjects was used. Cases were matched to controls for age (±5 years) and gender. A total of 330 AS patients and 330 controls provided written informed consent and were included in the analyses.

HLA-B27 status and *RANK*, *RANKL* and *OPG* gene polymorphisms

Peripheral blood was collected during medical surveillance, stored at 4°C and processed on the same day. The blood was centrifuged to separate the serum and cells. All specimens were stored at −70°C until analysis. Genomic DNA was extracted from peripheral blood using the AxyPrepTM Blood Genomic DNA Miniprep Kit (Axygen Scientific Corporation, Union City, CA). *HLA-B27* carriage was previously assessed by flow cytometry [28].

RANK C575T, *RANKL* C-290T and *OPG* G1181C genotypes were identified in all subjects using PCR-restriction fragment length polymorphism (PCR-RFLP). *RANK* genotype was determined according to Hsu *et al.* [18]. Primers used to amplify the *RANK* gene were 5′-CTC TGC TCC TTT GCT GAC CGC AAT CTC AGA-3′ and 5′-GCA GAG AAG AAC TGC AAA CCG CGT CGG-3′. DNA (0.5 μl) was added to a PCR buffer containing a 200 ng mixture of primers, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris–HCl (pH 8.3) and 0.1% BSA in a final volume of 50 μl. Amplification was carried out under the following conditions: denaturing was conducted for 1 min at 95°C, annealing for 1 min at 60°C and extension at for 1 min at 72°C. PCR products were digested with *Bst*UI for 16 h at 60°C. Homozygous TT individuals were those with two product fragments of 199 and 125 bp, homozygous CT individuals had a 199-, 104- and 21-bp fragment and heterozygous CC individuals had all four fragments. *RANKL* C-290T polymorphism was also determined using PCR-RFLP [17]. Primers used to amplify the *RANKL* gene were 5′-CAA GAT GCA GAA ATA GGG ATT TGG GAA GG-3′ and 5′-CTG GAG GTC CAA GAG ATG GGT TCA A-3′. Amplification was carried out as follows: denaturing was done for 1 min at 95°C, annealing for 1 min at 58°C and extension for 1 min at 72°C. PCR products were digested with *Hpy*188III for 20 h at 65°C. Homozygous TT individuals had a single-product fragment sized 294 bp, homozygous CC individuals had two fragments sized 269 and 25 bp and heterozygous CT individuals had all three fragments. All study subjects were observed to be *RANK* 575 C allele and *RANKL* −290 C allele. Determination of *OPG* polymorphism was done as previously described [22]. A Lys to Asn substitution in exon 1 (codon 3) was amplified to form a fragment of 147 bp using primers 5′-ACT TCC TGT TGC CGG GAC GCT A-3′ and 5′-TAC CAC GAG CGC GCA GCA CCT CA-3′. Amplification was carried out as follows: denaturing was done for 30 s at 95°C, annealing for 45 s at 60°C and extension for 30 s at 72°C. PCR products were digested with *Sml*I for 20 h at 55°C. Homozygous GG individuals

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had a single-product fragment sized 147 bp, homozygous CC individuals had two fragments sized 123 and 24 bp and heterozygous GC individuals had all three fragments. Direct sequencing of 10% of all subjects was done to validate PCR-RFLP.

Statistical analysis

There were no data about the frequency of *RANKL* C-290T and *RANK* C575T polymorphisms in the Taiwanese. Therefore, we only considered *OPG* G1181C genotype to calculate the sample size. Given a type I error (α) level of 0.05, type II error (β) level of 0.20, *OPG* G1181C GG genotype among healthy controls of 0.57 [21], detectable relative risks (RRs) of 2.0 and a control–case rate of 1.0, the minimum sample size required for cases is 266. As a few subjects might be lost to follow-up, we included additional subjects (~20%). Finally, we recruited 330 cases and 330 controls matched for age and gender.

Hardy–Weinberg equilibrium was tested by goodness-of-fit to compare the observed *OPG* genotypes with expected ones. The χ^2-test was also used to test the prevalence of *OPG* genotypes and alleles between case and control groups. The conditional logistic regression model was used for the matched RRs (RR_m) and 95% CI for *OPG* genotypes in AS patients and controls. Differences between genotypes and alleles in age of symptom onset, disease duration and delayed diagnosis were, respectively, calculated using analysis of variance (ANOVA) and the *t*-test to compare mean values. Differences in age of symptom onset among AS patients by *OPG* allele, *HLA-B27* and gender were determined by the *t*-test. Distribution of *OPG* genotypes in AS patients with or without peripheral arthritis, uveitis and IBD were tested by the χ^2-test or Fisher's exact test. A multiple unconditional logistic model was constructed to obtain the adjusted odds ratio (OR) for clinical manifestations and corresponding 95% CI for *OPG* genotypes after adjusting for potential confounding factors. *P*-values were adjusted for multiple comparisons by applying the Bonferroni–Holm procedure. All *P*-values were calculated using two-tailed statistical tests and a $P < 0.05$ was considered statistically significant. SAS 9.1 for Windows (SAS Inc., Cary, NC) was used for all analysis.

Results

The mean (S.D.) age of cases and controls was 43.7 (10.2) and 44.5 (10.6) years, respectively (Table 1). About 70% were male. In AS patients, 45.5% had a family history of AS. Mean age of symptom onset was 32.2 years, mean disease duration was 11.5 years and mean time from onset to diagnosis was 5.5 years. Of AS patients, 46.1% were diagnosed with peripheral arthritis, 28.2% with uveitis and 4.5% with IBD. In total, 91.5% ($n = 302$) of AS patients were *HLA-B27*$^+$.

The genotype and allele frequencies of *RANK*, *RANKL* and *OPG* in study subjects are shown in Table 2. All subjects had *RANK* 575C and *RANKL* −290C alleles. Distribution of *OPG* G1181C GG, GC and CC genotypes among controls were 57.6, 38.2 and 4.2%, respectively, and conformed to Hardy–Weinberg equilibrium. The number of *OPG* CC genotypes was small and were therefore combined with *OPG* GC in subsequent analysis. Individuals with the *OPG* homozygous GG genotype had a 1.74-fold (95% CI 1.26, 2.40) greater risk of AS compared with those with the GC and CC genotypes. *OPG* G allele was also associated with a higher AS risk than *OPG* C allele ($RR_m = 1.59$; 95% CI 1.21, 2.09).

In Table 3, we analysed differences in age of symptom onset, disease duration and delayed diagnosis in AS patients by *OPG* genotype and allele. Mean age of symptom onset was the oldest in *OPG* GG [34.0 (12.6) years] *vs* GC [28.4 (10.6) years] or CC [23.8 (7.4) years] genotypes ($P < 0.001$). Greater age of symptom onset was also observed in *OPG* G allele carriers.

Age of symptom onset also differed in AS patients by gender [male: 31.1 (12.8) *vs* female: 34.7 (10.6), $P = 0.010$; *t*-test] and *HLA-B27* [positive: 31.6 (12.1) *vs*

Table 1 Basal and clinical characteristics of AS patients and controls

Characteristic	AS cases	Controls
Number of subjects	330	330
Age, years	43.7 (10.2)	44.5 (10.6)
Gender: male, *n* (%)	230 (69.7)	230 (69.7)
Family history, *n* (%)	150 (45.5)	
Clinical features		
Age on initial symptom, years	32.2 (12.3)	
Disease duration, years	11.5 (10.4)	
Delayed diagnosis, years	5.5 (7.6)	
Peripheral arthritis, *n* (%)	152 (46.1)	
Uveitis, *n* (%)	93 (28.2)	
IBD, *n* (%)	15 (4.5)	

Data represent mean (S.D.) unless otherwise specified.

Table 2 Association of genotype and allele frequency of *RANK*, *RANKL* and *OPG* and AS occurrence

Variable	AS cases (%)	Controls (%)	RR_m (95% CI)	*P*-value
RANK C575T genotype				
CC	330 (100.0)	330 (100.0)	–	
RANKL C-290T genotype				
CC	330 (100.0)	330 (100.0)	–	
OPG G1181C genotype				
GG	232 (70.3)	190 (57.6)*	2.14 (0.88, 5.19)	0.095
GC	90 (27.3)	126 (38.2)	1.25 (0.50, 3.10)	0.630
CC	8 (2.4)	14 (4.2)	1.00 (reference)	
GG	232 (70.3)	190 (57.6)**	1.74 (1.26, 2.40)	<0.001
GC/CC	98 (29.7)	140 (42.4)	1.00 (reference)	
Allele				
G	554 (83.9)	506 (76.7)***	1.59 (1.21, 2.09)	0.001
C	106 (16.1)	154 (23.3)	1.00 (reference)	

*$P = 0.003$, χ^2-test was used to test the prevalence of *OPG* genotypes in AS patients and controls. **$P < 0.001$, χ^2-test was used to test the prevalence of *OPG* genotypes in AS patients and controls. ***$P < 0.001$, χ^2-test was used to test the prevalence of *OPG* alleles in AS patients and controls.

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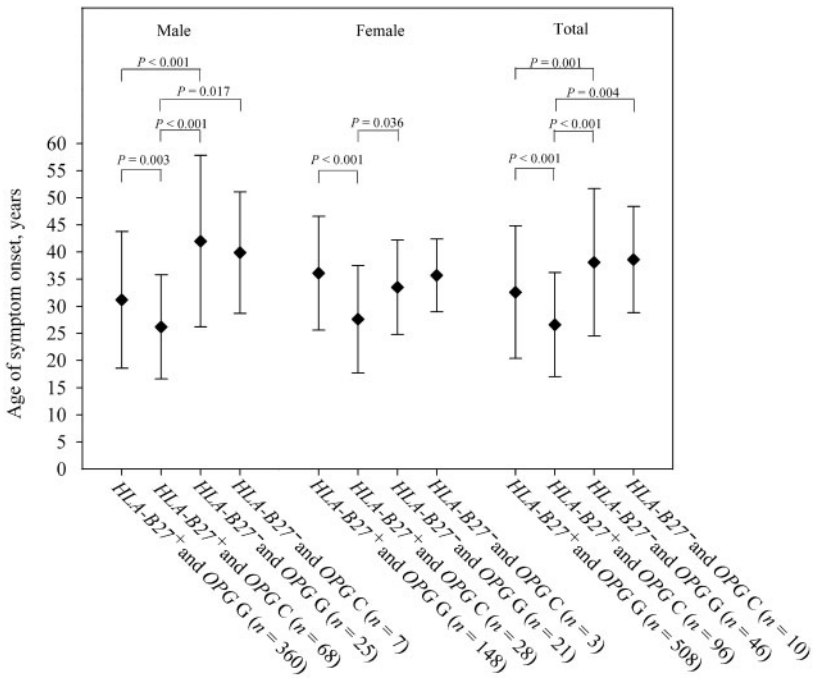
Table 3 Clinical features of AS patients by *OPG* G1181C genotype

Variable	Genotypes			Alleles	
	GG	GC	CC	G	C
No. of subjects	232	90	8	554	106
Age of symptom onset, years	34.0 (12.6)	28.4 (10.6)	23.8 (7.4)*	33.1 (12.4)	27.7 (10.2)**
Disease duration, years	11.4 (10.6)	12.0 (9.9)	9.6 (8.0)***	11.5 (10.5)	11.7 (9.6)****
Delayed diagnosis, years	5.5 (7.7)	5.5 (7.5)	4.9 (8.0)*****	5.5 (7.7)	5.4 (7.5)******

Data represent mean (S.D.) unless otherwise specified. *$P = 0.011$, ANOVA (adjusted $P = 0.033$, Bonferroni–Holm test). **$P < 0.001$, *t*-test (adjusted *P*-value = 0.003, Bonferroni–Holm test). ***$P = 0.767$, ANOVA. ****$P = 0.560$, *t*-test. *****$P = 0.972$, ANOVA. ******$P = 0.955$, *t*-test.

Fig. 1 Differences in age of symptom onset in AS patients stratified by *OPG* allele, *HLA-B27* and gender. Data represent mean (S.D.).



negative: 38.2 (13.0), $P = 0.007$]. Therefore, we analysed age of symptom onset among AS patients by *HLA-B27*, *OPG* allele and gender (Fig. 1). Individuals with *HLA-B27*$^+$ and *OPG* C allele had the earliest symptom onset [26.6 (9.6) years], followed by *HLA-B27*$^+$ and *OPG* G allele [32.6 (12.2) years], *HLA-B27*$^-$ and *OPG* G allele [38.1 (13.6) years] and *HLA-B27*$^-$ and *OPG* C allele [38.6 (9.8) years]. Compared with *HLA-B27*$^+$ and *OPG* C allele patients, age of symptom onset in other groups was significantly later (*P*'s < 0.005). In AS males, *HLA-B27*$^+$ and *OPG* C allele was associated with the least age of symptom onset [26.2 (9.6) years], followed by *HLA-B27*$^+$ and *OPG* G allele [31.2 (12.6) years], *HLA-B27*$^-$ and *OPG* C allele [39.9 (11.2) years] and *HLA-B27*$^-$ and *OPG* G allele [42.0 (15.8) years]. Similar results were observed in females. When multiple testing (Bonferroni–Holm correction) was taken into consideration, *HLA-B27*$^+$ and *OPG* C allele patients remained a significant earlier age of symptom onset (*P*'s < 0.01).

Distribution of *OPG* genotypes in AS patients with or without peripheral arthritis, uveitis and IBD is shown in Table 4. The frequency of peripheral arthritis in AS patients with *OPG* GG, GC and CC genotypes were 41.8, 60.0 and 12.5%, respectively. The prevalence of *OPG* genotypes in the groups of AS patients with or without peripheral arthritis differed significantly ($P = 0.001$, Fisher's exact test). Since few carried the *OPG* CC genotype, we combined *OPG* GC and CC genotypes in further analysis. After adjusting for the effects of age, gender and age of symptom onset, we found that *OPG* homozygous GG carriers had a 1.53-fold (95% CI 0.92, 2.55) greater risk of peripheral arthritis compared with *OPG* GC and CC carriers. However, no associations were observed between *OPG* genotypes and development of uveitis or IBD among AS patients.

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Discussion

This study found the *OPG* G1181C genetic polymorphism associated with AS development. Age of symptom onset and development of peripheral arthritis in AS patients differed significantly by *OPG* genotype.

Osteoporosis and fractures of the vertebral body are recognized features of AS [5, 6]. Bone loss is caused by greater bone resorption than bone formation [7]. OPG produced by osteoblasts binds to RANKL, regulating bone resorption by inhibiting osteoclast differentiation and activation and inducing their apoptosis [12–14]. Franck *et al.* [29] reported that BMD of the hip and femoral neck were significantly lower in AS patients than in controls, and bone loss in AS patients may be associated with lower serum OPG levels. A G to C polymorphism at position 1181 in the first exon of the *OPG* gene results in an amino acid substitution of Asn for Lys [21]. In the Chinese postmenopausal women, BMD at the lumbar spine was significantly higher in *OPG* CC *vs* GC and GG genotypes. Subjects with *OPG* GG genotype have greater risk for osteoporosis than those with the CC genotype [21]. Langdahl *et al.* [22] found that the CC genotype is less common among those with osteoporosis than controls, and that those with the CC genotype in both groups had greater BMD compared with other genotypes. However, studies looking for an association between the *OPG* G1181C genotype and osteoporosis were inconsistent [30, 31]. The Irish postmenopausal women carrying the C allele had lower BMD at the lumbar spine and the femoral neck than those carrying the G allele [30]. However, Ueland *et al.* [31] reported the *OPG* G1181C genotype and OPG serum levels were not associated with osteoporosis. Differences in frequency of *OPG* G1181C genotypes by ethnicity may cause this inconsistency. Frequencies of the C allele in the Irish and Australian subjects were 37.3 and 52.1%, respectively [30, 31]. In the present study, the *OPG* C allele frequency in controls was 23.3%, in conformity with Hardy–Weinberg equilibrium. This was comparable with corresponding values among controls in the Chinese (26%) and the Japanese (28%) populations [21]. Such results reinforce our genotyping techniques. The relationship of the *OPG* gene and osteoporosis has yet to be elucidated. We observed that *OPG* GG subjects had a 1.74-fold risk for AS compared with GC and CC, and the *OPG* G1181C allele showed evidence of an allele dose effect. Therefore, our result suggests that the *OPG* G allele was less able to bind with RANKL than the *OPG* C allele, leading to increased bone resorption and AS development. However, functional studies are required to test this association.

In addition to occurrence, severity and age of symptom onset of AS may also be associated with genetic traits [32]. In the multiplex families of SpA, cluster analysis revealed that age of symptom onset was associated with inheritance, independent of disease duration and may be determined by specific genetic factors [24]. The *HLA-B27* gene is known for its strong association with AS [3]. An early study has demonstrated that *HLA-B27* gene is associated with earlier onset of AS symptoms [33]. A previous study in the Han Chinese also found that *HLA-B27*$^+$ AS patients had first symptoms earlier than *HLA-B27*$^-$ patients [34]. Our results were similar. A possible explanation is that the antigenic fragments of *HLA-B27* gene may be wrongly identified by the immune system, causing *HLA-B27*$^+$ subjects to progress more swiftly [34]. We also found that the *OPG* G1181C genotype correlated with the age of symptom onset in AS patients. Furthermore, *HLA-B27*$^+$ patients with the *OPG* C allele had the earliest age of symptom onset, a mere 26.6 years. In our observation, *OPG* C allele is protective in AS development. Therefore, AS development in *OPG* C allele patients may be influenced mainly by *HLA-B27* status. Our *HLA-B27*$^-$ AS patients with *OPG* G allele had the oldest age of symptom onset (38.1 years). However, *OPG* G allele was considered as a risk factor for AS development in our subjects. Therefore, AS development in *HLA-B27*$^-$ patients with *OPG* G allele might be induced by *OPG* G allele, without regard to *HLA-B27* status. Interestingly, *HLA-B27*$^+$ AS patients with *OPG* G allele had the second-earliest age on initial symptom (32.6 years). AS development in *HLA-B27*$^+$ patients with *OPG* G allele might be induced by both *OPG* G allele and *HLA-B27*, separately. Early-stage *HLA-B27*$^+$ subjects without AS might develop osteoporosis induced by the *OPG* gene and then, later, AS. Finally, *HLA-B27*$^-$ patients with *OPG* C allele had the latest age of symptom onset. Pathogenesis in these patients may be induced by neither *HLA-B27* nor *OPG* G allele, but by other risk factors.

In our study, the age of symptom onset in AS patients with different *OPG* alleles and *HLA-B27* was further compared by gender. Similar distribution in age of symptom onset was observed in male and female patients. However, the lack of female patients may have limited statistical power. Sex steroid hormones, parathyroid hormone and vitamin D may contribute to the bone loss associated with AS [29], but we did not evaluate these factors. Overall, *HLA-B27* and *OPG* may contribute to variations in AS patients, with the *HLA-B27* gene contributing to earlier onset of symptom and the *OPG* gene inducing later osteoporosis.

Patients with AS may not only display axial involvement but also progress to peripheral joint involvement [35]. Previous studies suggest that bone loss in AS patients is associated with concentration of bone resorption markers modulated by the RANKL/OPG system [36, 37] and enhanced inflammatory activity [38]. Since approximately half of our patients experienced peripheral arthritis, it was used to assess disease progression. We found occurrence of peripheral arthritis differed significantly by *OPG* G1181C genotype, with AS patients with *OPG* GG genotype having a higher, though insignificant, risk for peripheral arthritis than those with *OPG* GC and CC genotypes. Others have found expression of osteoclasts infiltrating the periarticular cortical bone associated with erosion of the subchondral bone [39]. Abundant RANK, RANKL and OPG expression have also been found to be partly independent of inflammation [40]. Our result might reflect the

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TABLE 4 Distribution of *OPG* genotypes in AS cases with and without peripheral arthritis, uveitis and IBD

OPG genotype	GG $n=232$, n (%)	GC $n=90$, n (%)	CC $n=8$, n (%)	GG OR (95% CI)[a]	GC and CC Reference
Peripheral arthritis					
Yes	97 (41.8)	54 (60.0)	1 (12.5)[b,*]	1.53 (0.92, 2.55)	1.00
No	135 (58.2)	36 (40.0)	7 (87.5)		
Uveitis					
Yes	61 (26.3)	31 (34.4)	1 (12.5)[b,**]	1.35 (0.78, 2.32)	1.00
No	171 (73.7)	59 (65.6)	7 (87.5)		
IBD					
Yes	9 (3.9)	6 (6.7)	0 (0.0)[b,***]	1.73 (0.57, 5.26)	1.00
No	223 (96.1)	84 (93.3)	8 (100.0)		

[a]A multiple unconditional logistic model was constructed to obtain the adjusted OR for clinical manifestations and corresponding 95% CI for *OPG* genotypes after adjusting for potential age, gender and age of symptom onset. [b]Distribution of *OPG* genotypes in AS patients with or without peripheral arthritis, uveitis and IBD were tested by Fisher's exact test. *$P=0.001$, Fisher's exact test (adjusted $P=0.006$, Bonferroni–Holm test). **$P=0.249$, Fisher's exact test. ***$P=0.571$, Fisher's exact test.

poor binding of OPG protein with RANKL in AS patients with *OPG* G allele, causing osteoclast proliferation and peripheral arthritis development. However, the influence of NSAIDs and DMARDs was not evaluated, suggesting that studies to assess such drugs are warranted.

RANK C575T and *RANKL* C-290T polymorphisms were not detected in our subjects. Kim *et al.* [41] also found no *RANK* C575T polymorphism in the Korean postmenopausal women. However, future studies should evaluate the role of other *RANK* and *RANKL* polymorphisms in AS development. This study had certain limitations. Selection bias may have occurred when AS patients with active or inactive disease were enrolled. Since our subjects were adults, results do not apply to juvenile AS patients. Referral bias was possible, since our data were collected from a single medical centre. Although recall bias is possible, our use of three instruments made this unlikely. In addition, there were no data on the bone status (such as the incidence of vertebral and peripheral fractures, bone density measurements and bone turnover markers) of the patients.

In summary, subjects with the *OPG* G1181C G allele had greater risk of AS than those with *OPG* C allele. The *HLA-B27* gene is associated with earlier onset of AS symptoms, whereas the *OPG* gene is associated with induction of later osteoporosis.

Rheumatology key messages

- The *HLA-B27* gene may be associated with earlier onset of AS symptoms.
- The *OPG* gene may be associated with induction of later osteoporosis in AS patients.

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References

1 Braun J, Sieper J. Ankylosing spondylitis. Lancet 2007; 369:1379–90.
2 Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. Lancet 1999;354: 1687–90.
3 Brewerton DA, Hart FD, Nicholls A *et al*. Ankylosing spondylitis and HL-A 27. Lancet 1973;1:904–7.
4 Khan MA, Ball EJ. Genetic aspects of ankylosing spondylitis. Best Pract Res Clin Rheumatol 2002;16: 675–90.
5 Ralston SH, Urquhart GD, Brzeski M *et al*. Prevalence of vertebral compression fractures due to osteoporosis in ankylosing spondylitis. Br Med J 1990;300:563–5.
6 Donnelly S, Doyle DV, Denton A *et al*. Bone mineral density and vertebral compression fracture rates in ankylosing spondylitis. Ann Rheum Dis 1994;53:117–21.
7 Hadjidakis DJ. Androulakis II. Bone remodeling. Ann N Y Acad Sci 2006;1092:385–96.
8 Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. Arthritis Res Ther 2007;9(Suppl. 1):S1.
9 Yasuda H, Shima N, Nakagawa N *et al*. A novel molecular mechanism modulating osteoclast differentiation and function. Bone 1999;25:109–13.
10 Hsu H, Lacey DL, Dunstan CR *et al*. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999; 96:3540–5.

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11 Simonet WS, Lacey DL, Dunstan CR *et al*. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.

12 Lacey DL, Timms E, Tan HL *et al*. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.

13 Burgess TL, Qian Y, Kaufman S *et al*. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol 1999;145:527–38.

14 Akatsu T, Murakami T, Nishikawa M *et al*. Osteoclastogenesis inhibitory factor suppresses osteoclast survival by interfering in the interaction of stromal cells with osteoclast. Biochem Biophys Res Commun 1998;250:229–34.

15 Mizuno A, Amizuka N, Irie K *et al*. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 1998;247:610–5.

16 Wong BR, Rho J, Arron J *et al*. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997;272:25190–4.

17 Mencej S, Prezelj J, Kocijancic A *et al*. Association of TNFSF11 gene promoter polymorphisms with bone mineral density in postmenopausal women. Maturitas 2006;55:219–26.

18 Hsu YH, Niu T, Terwedow HA *et al*. Variation in genes involved in the RANKL/RANK/OPG bone remodeling pathway are associated with bone mineral density at different skeletal sites in men. Hum Genet 2006;118:568–77.

19 Wuyts W, Van Wesenbeeck L, Morales-Piga A *et al*. Evaluation of the role of RANK and OPG genes in Paget's disease of bone. Bone 2001;28:104–7.

20 Morinaga T, Nakagawa N, Yasuda H *et al*. Cloning and characterization of the gene encoding human osteoprotegerin/osteoclastogenesis-inhibitory factor. Eur J Biochem 1998;254:685–91.

21 Zhao HY, Liu JM, Ning G *et al*. The influence of Lys3Asn polymorphism in the osteoprotegerin gene on bone mineral density in Chinese postmenopausal women. Osteoporos Int 2005;16:1519–24.

22 Langdahl BL, Carstens M, Stenkjaer L *et al*. Polymorphisms in the osteoprotegerin gene are associated with osteoporotic fractures. J Bone Miner Res 2002;17:1245–55.

23 Hamersma J, Cardon LR, Bradbury L *et al*. Is disease severity in ankylosing spondylitis genetically determined? Arthritis Rheum 2001;44:1396–400.

24 Porcher R, Said-Nahal R, D'Agostino MA *et al*. Two major spondylarthropathy phenotypes are distinguished by pattern analysis in multiplex families. Arthritis Rheum 2005;53:263–71.

25 Breban M, Miceli-Richard C, Zinovieva E *et al*. The genetics of spondyloarthropathies. Joint Bone Spine 2006;73:355–62.

26 Maksymowych WP, Russell AS. Polymorphism in the LMP2 gene influences the relative risk for acute anterior uveitis in unselected patients with ankylosing spondylitis. Clin Invest Med 1995;18:42–6.

27 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.

28 Chou CT, Tsai YF, Liu J *et al*. The detection of the HLA-B27 antigen by immunomagnetic separation and enzyme-linked immunosorbent assay-comparison with a flow cytometric procedure. J Immunol Methods 2001;255:15–22.

29 Franck H, Meurer T, Hofbauer LC. Evaluation of bone mineral density, hormones, biochemical markers of bone metabolism, and osteoprotegerin serum levels in patients with ankylosing spondylitis. J Rheumatol 2004;31:2236–41.

30 Chou CT, Tsai YF, Liu J *et al*. Investigation of the genetic influence of the OPG, VDR (Fok1), and COLIA1 Sp1 polymorphisms on BMD in the Irish population. Calcif Tissue Int 2002;71:26–35.

31 Ueland T, Bollerslev J, Wilson SG *et al*. No associations between OPG gene polymorphisms or serum levels and measures of osteoporosis in elderly Australian women. Bone 2007;40:175–81.

32 Brophy S, Calin A. Ankylosing spondylitis: interaction between genes, joints, age at onset, and disease expression. J Rheumatol 2001;28:2283–8.

33 Khan MA, Kushner I, Braun WE. Comparison of clinical features in HLA-B27 positive and negative patients with ankylosing spondylitis. Arthritis Rheum 1977;20:909–12.

34 Wu Z, Lin Z, Wei Q *et al*. Clinical features of ankylosing spondylitis may correlate with HLA-B27 polymorphism. Rheumatol Int 2009;29:389–92.

35 Ozgocmen S, Godekmerdan A, Ozkurt-Zengin F. Acute-phase response, clinical measures and disease activity in ankylosing spondylitis. Joint Bone Spine 2007;74:249–53.

36 MacDonald AG, Birkinshaw G, Durham B *et al*. Biochemical markers of bone turnover in seronegative spondylarthropathy: relationship to disease activity. Br J Rheumatol 1997;36:50–3.

37 Hofbauer LC, Heufelder AE. The role of osteoprotegerin and receptor activator of nuclear factor kappaB ligand in the pathogenesis and treatment of rheumatoid arthritis. Arthritis Rheum 2001;44:253–9.

38 Broman P, Bodur H, Bingöl N *et al*. Bone mineral density and bone turnover markers in a group of male ankylosing spondylitis patients: relationship to disease activity. J Clin Rheumatol 2001;7:315–21.

39 Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum 1984;27:968–75.

40 Vandooren B, Cantaert T, Noordenbos T *et al*. The abundant synovial expression of the RANK/RANKL/Osteoprotegerin system in peripheral spondylarthritis is partially disconnected from inflammation. Arthritis Rheum 2008;58:718–29.

41 Kim JG, Kim JH, Kim JY *et al*. Association between osteoprotegerin (OPG), receptor activator of nuclear factor-kappaB (RANK), and RANK ligand (RANKL) gene polymorphisms and circulating OPG, soluble RANKL levels, and bone mineral density in Korean postmenopausal women. Menopause 2007;14:913–8.

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