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].
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 TTG GAT 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 MgCl₂, 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 BstUI 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 CT 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 AAA TGC AAA CCG CGT CGG-3' and 5'-GTA GAG AAG AAC TGC AAA CCG CGT CGG-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 Hpy188III 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 RANKL 575 T allele and RANKL C-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 GGC 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 Smal for 20 h at 55°C. Homozygous GG individuals
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 $\chi^2$-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 (Rm) 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 HLA-B27 genotype 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 $\chi^2$-test or Fisher’s exact test. A multiple unconditional logistic regression 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 (Rm = 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 negative: 34.0 (12.2), **$P = 0.001$**]. Mean age of symptom onset also differed in AS patients by disease duration and delayed diagnosis were, respectively, 11.5 (10.4) and 5.5 (7.6) years. Of AS patients, 46.1% 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*.

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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 (\( P' < 0.005 \)). 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 (\( P' < 0.01 \)).

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.

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

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

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. ****\( P = 0.972 \), ANOVA. *****\( P = 0.955 \), t-test.
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
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.

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Disclosure statement: The authors have declared no conflicts of interest.

References

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

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

* 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. **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). \(b^{***}\) P = 0.249, Fisher’s exact test. \(b^{***}\) P = 0.571, Fisher’s exact test.

Acknowledgements
We thank the clinical nurses of clinical trail centre, Chung Shan Medical University, for their kind assistance in recruiting patients.

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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