Single Nucleotide Polymorphisms of TRAF2 and TRAF5 Gene in Ankylosing Spondylitis: A Case-Control Study

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Research Article

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Abstract

Objective

To investigate the role of eight locus polymorphisms of tumor necrosis factor receptor associated factor 2 (TRAF2) and TRAF5 gene and their interaction in the susceptibility to ankylosing spondylitis (AS) in Chinese Han population.

Methods

Eight single nucleotide polymorphisms (SNPs) (rs3750511, rs10781522, rs17250673, rs59471504, rs6540679, rs12569232, rs4951523, rs7514863) of TRAF2 and TRAF5 gene were genotyped in 673 AS patients and 687 controls.

Results

The SNPs of TRAF2 and TRAF5 does not indicate a correlation with the susceptibility of AS in Chinese Han population. Genotype frequencies of rs3750511 were statistically significant in females between patients and controls. The genotype frequencies of rs12569232 and allele frequencies of rs3750511 were statistically significant between groups of different diseases activity. One three-locus model, TRAF2 (rs10781522, rs17250673) and TRAF5 (rs12569232), had a maximum testing accuracy of 52.67% and a maximum cross-validation consistency (10/10) that was significant at the level of P = 0.0001, after determined empirically by permutation testing. As to environmental variables, only marginal association was found between sleep quality and AS susceptibility.

Conclusion

TRAF2 rs3750511 polymorphism may be associated with the susceptibility and severity of AS. Besides, the interaction of TRAF2 and TRAF5 genes may be associated with AS susceptibility, but many open questions remain.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease, which first affects the sacroiliac joints and the spinal osteoid process joint [1, 2]. AS usually occurs in young people between 20 and 30 years old, and the incidence rate in men is higher than that in women, with a ratio of about 2:1 [3]. In addition, AS has insidious onset, slow progression and high disability rate, which seriously affects the working ability of the patients and increases the social burden [4].

There is currently no cure for ankylosing spondylitis, but it can be alleviated with surgery, medication, physical therapy, and exercise [5-7]. Among them, biological agents such as tumor necrosis factor-α (TNF-α) inhibitors are the best choice for the treatment of ankylosing spondylitis, which has good therapeutic effect and can effectively slow the disease progression, but the cost is relatively high.

Up to now, the pathogenesis of AS remains unclear. Most researchers believe that its occurrence may be related to genetic predisposition, environmental exposure, immunity and other factors. A large number of studies have shown that the occurrence of the disease is closely related to the human leukocyte antigen (HLA) region gene represented by the HLA-B27 gene [8, 9], and 90% of AS patients are HLA-B27 gene positive [10]. However, the twin study has found that HLA-B27 can only explain 20% of the genetic susceptibility to AS [11], suggesting that there may be other factors involved in the incidence of AS.

TNF-α is a multifunctional cytokine, which is abundant in the sacroiliac joint of AS patients, and has been shown to play an important role in the pathogenesis and development of AS [12, 13]. TNF receptor associated factor 2 (TRAF2) and TRAF5 gene, as members of the TNF receptor-related factor family, could be expressed in various immune cells, such as macrophages and lymphocytes, and could regulate inflammatory cytokines such as TNF-α and interleukin (IL)-1 [14]. TRAF2 and TRAF5, acts as the signal transducer, link members of the TRAF family to different signaling pathways, such as regulating nuclear factor kappa-B (NF-κB) and Toll-like receptors (TLR) activation [15]. It has been shown that TRAF2 and TRAF5 are indispensable in the NF-κB signaling pathway [16]. Meanwhile, NF-κB is considered as a common transcription factor that is critical for innate and adaptive immunity, and has been implied to play a role in autoimmune and auto-inflammatory diseases, such as rheumatoid arthritis (RA) [14], juvenile idiopathic arthritis (JIA) [17] and AS [18].

Single nucleotide polymorphism (SNP) is the difference of single base in the DNA sequence of different individuals and is a common type of human heritable variation [19]. A series of studies have shown that SNPs of TRAF2 and TRAF5 were associated with a variety of autoimmune diseases, such as acute anterior uveitis (AAU) and RA [14, 16]. The study showed that 20% to 40% of AS patients will have an episode of AAU in the course of their disease [20]. Both AS and AAU were associated with the HLA-B27 genotype [21] and the pathophysiologic similarities between AS and AAU have been extensively studied [22]. The relationship between TRAF2 and TRAF5
polymorphisms and AS has not been reported in domestic and foreign. In this study, we explored the role of eight tag SNPs of TRAF2 and TRAF5 gene and their interaction in the susceptibility to AS in Chinese Han population, which will help to further understand the pathogenesis of AS and provide a basis for clinical targeted therapy.

2. Material And Methods

2.1 Subjects

A case-control association study was used to investigate the role of TRAF2 and TRAF5 polymorphisms in AS susceptibility. We obtained approval of the study protocol from the Ethical Committee of Anhui Medical University (Hefei, China) and all procedures have complied with the 1964 Declaration of Helsinki. All the subjects were given an informed consent and were well told of the study protocol. All participants were genetically unrelated Chinese. AS patients were recruited from outpatient clinics at the First Affiliated Hospital of Anhui Medical University, Hefei, China. All patients were diagnosed by the skilled rheumatologist according to the modified 1984 New York Criteria. Healthy controls with no history of AS were recruited from healthy blood donors. AS patients and healthy controls (HCs) were excluded from the present study if they complicated with RA, AAU, IBD, pulmonary tuberculosis, systemic lupus erythematosus, psoriatic arthritis, psoriasis, or other chronic inflammatory or immune diseases. Healthy controls were gender, age and ethnicity matched to the patients. 673 AS patients and 687 controls were recruited from March 2011 to September 2019. Body mass index (BMI), HLA-B27, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), disease duration, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Ankylosing Spondylitis Disease Activity Score (ASDAS) and environmental factors (smoking, drinking, salt intake level, cooking oil, frequency of eating fatty meat, frequency of drinking milk, type of drinking water, noise, sleep quality, damp condition of residence, frequency of exercise) of all patients were recorded by using a structured questionnaire. Both BASFI and BASDAI are visual self-report scales (0–10 cm), ASDAS was calculated by combining multiple factors, and a higher score indicates high disease severity.

2.2 SNPs selection and genotyping

Four tag SNPs (rs3750511, rs10781522, rs10781522 and rs59471504) in TRAF2 and four tag SNPs (rs6540679, rs12569232, rs4951523 and rs7514863) in TRAF5 were selected by using the Tagger program in Haploview 4.2 (Broad Institute, Cambridge, MA, USA) in the context of the HapMap databases in the Chinese Han population in Beijing (CHB) (HapMap Data Rel 28 Phasell+III, 10 August, on NCBI B36 assembly, dpSNP b126). Tag SNPs were identified as candidate SNPs to cover polymorphisms with minimum minor allele frequency ⩾ 5% in TRAF2 and TRAF5 gene with an \( r^2 \) of 0.80 or greater. Selection of SNPs was completed in January 2018. Genomic DNA was extracted from peripheral blood lymphocytes using a commercially available kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA samples were stored at −80 °C before genotyping. The genotyping of SNPs was carried out using the improved Multiple Ligase Detection Reaction (iMLDR) Assay technology by Shanghai Genesky Bio-Tech Co., Ltd (http://biotech.geneskies.com/index.html). The primers are listed in Table S1. Raw data were analyzed by using GeneMapper 4.1 (Applied Biosystems, Foster City, CA, USA).

2.3 Statistical analysis

Data analysis was performed in SPSS 23.0 (SPSS, Chicago, IL, USA). Quantitative data was presented as mean ± standard deviation (SD) or median and inter-quartile range (IQR), while qualitative data was expressed by percentages. Normal distributions were tested with the Kolmogorov–Smirnov test with Lilliefors correction. Continuous variables of AS and HCs were compared by Student's t-test or Mann-Whitney U test when appropriate. The statistical differences between AS and HCs in allele, genotypes and genetic models were assessed by Chi-square test or Fisher's exact test. Additionally, subgroup analysis based on gender, HLA-B27 and clinical characteristics was also conducted for further information. Hardy-Weinberg equilibrium (HWE) tests were performed in healthy controls by the Chi-square test. Logistic regression analysis was performed to explore the association between genes and environmental factors. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated. The gene-gene interaction was analyzed by multifactor dimensionality reduction (MDR) [23]. MDR combined high and low propensity genotypes into two different groups (high or low risk). Then the combined model was selected based on the lower misclassification error. And by calculating the prediction error, the models were cross-verified by 10 times. Then, the best model with the greatest cross-validation consistency was selected. The \( P \) value of prediction accuracy was empirically determined by permutations of case and control labels for 1000 times. Using the hierarchical interaction diagram and interaction tree diagram of MDR, the single nucleotide polymorphism interaction of the optimal model was given [24]. In addition, traditional statistical methods were used to test the MDR analysis results. All statistical tests were two sides and \( P < 0.05 \) was considered
to be statistically significant. And Bonferroni correction was used for the correction for multiple comparisons. \( P \)-value for a truly significant result was set at 0.05/n, where \( n \) indicates the number of comparisons.

### 3. Results

#### 3.1 Characteristics of study subjects

The SNPs group consisted of 673 unrelated AS patients (548 males and 125 females) and 687 unrelated healthy controls (560 males and 127 females). The mean age of AS and HCs was 28.58 ± 9.31 and 28.62 ± 7.76 years respectively. No statistically significant differences were observed between the two groups regarding gender (\( \chi^2 = 0.002, P = 0.967 \)) and age (\( t = 0.087, P = 0.931 \)). Specific clinical characteristics of AS patients were depicted in Table 1.

#### 3.2 Genotype, allele and inheritance models analysis

All of the SNPs were in Hardy–Weinberg equilibrium in control group (all \( P > 0.05 \)). The minor allele frequencies in our study were consistent with the International HapMap Project data for Chinese Han population in Beijing (CHB) (Table S1). The genotype and allelic frequencies of each tag SNP were compared between patients and controls, but no significant associations were identified (all \( P > 0.05 \), details in Table 2).

The results of subgroup analysis in gender showed that in the female population, the rs3750511 genotype frequency distribution was statistically significant between the case group and the control group (\( c^2 = 5.907, P = 0.033 \)), but not statistically significant after Bonferroni correction (\( P > 0.05/2 = 0.025 \)). The genotype frequencies of rs10781522 were statistically significant between BASDAI<4 group and BASDAI\( \geq \)4 group (\( c^2 = 4.434, P = 0.035 \)), but the difference was not statistically significant after Bonferroni correction (\( P > 0.05/2 = 0.025 \)). The genotype frequencies of rs3750511 were statistically significant between BASDAI<4 group and BASDAI\( \geq \)4 group (\( c^2 = 10.962, P = 0.004 \)), and the difference was still statistically significant after Bonferroni correction (\( P < 0.05/2 = 0.025 \)). In other subgroup analysis based on gender, HLA-B27, BASFI and BASDAI, there were no significant differences in alleles and genotypes of eight SNPs between groups (all \( P > 0.05 \); Table 2 and Table 3).

Furthermore, dominant and recessive models were conducted between AS patients and healthy controls. No significant relationships were identified under dominant and recessive models or in the stratification analysis by gender (not shown).

#### 3.3 Gene-gene interactions

The distribution of high-risk and low-risk genotypes in the best three-locus model were shown in Fig. 1 respectively. The results of the cross-validation consistency and prediction error of each locus obtained by MDR analysis were shown in Table 4. After the best three-locus model \([\text{TRAF2 (rs10781522, rs17250673) and TRAF5 (rs12569232)}]\) were determined by the replacement test, at the test level \( P = 0.001 \), the maximum test accuracy was 52.67\%, and the maximum cross-validation consistency was 10/10.

#### 3.4 Gene-environment association analysis

There was an association between of rs3750511 polymorphism and sleep quality in the dominant model (OR: 2.446, 95\%CI: 1.060-5.643; \( P = 0.036 \)) (Table 5). No statistical differences were found in other SNPs or gene models, and the results were not provided.

### Discussion

TRAF2 and TRAF5, members of the TRAF family, could activate downstream intracellular signaling cascades through its cell surface receptors. Meanwhile, as non-HLA region genes, TRAF2 and TRAF5 are highly polymorphic. Therefore, it is necessary to explore the association between TRAF2 and TRAF5 gene polymorphisms and the susceptibility to ankylosing spondylitis.

This study showed that, rs3750511 of TRAF2 gene may be the susceptibility site of AS in Chinese Han female population, which further indicates that TNF signaling may increase AS susceptibility, especially in female population. For the difference in gender susceptibility, the most likely cause was usually considered as sex hormones, which were important regulators of the immune response process [25]. To investigate whether TRAF2 and TRAF5 gene polymorphisms are not only associated with the occurrence of disease, but also with disease development and disease activity, we conducted the stratified analysis based on disease activity indicators. The results indicated that the allele frequencies of rs10781522 and the genotype frequencies of rs3750511 were statistically significant between groups of BASDAI < 4 and BASDAI \( \geq \) 4. The above results indicated that rs10781522 and rs3750511 polymorphism may be associated with disease activity in
AS. TRAF2, a member of the TRAF family, could activate the C-JNK and IKK pathways, which in turn induce the expression of genes involved in inflammation, immune response, cell proliferation, cell differentiation, and inhibition of death receptor-induced apoptosis [14]. Meanwhile, some studies reported TRAF2 gene expression was related to the level of TNF-α, which may explain the association between the SNPs of TRAF2 gene and the disease activity of AS.

Numerous studies showed that there were common genetic pathways and immune mechanisms between ankylosing spondylitis and inflammatory bowel disease and rheumatoid arthritis. And it was found that rs7514863 SNP on TRAF5 gene was associated with rheumatoid arthritis [14]. However, in this study, we found no significant differences in the distribution of genotype frequency, allele frequency and inheritance model of rs7514863 between AS patients and healthy controls. The reason for this difference may be due to ethnic differences, the same genetic locus of the same disease may also have different results depending on ethnicity. In addition, although the genetic pathway of ankylosing spondylitis has many similarities with rheumatoid arthritis, it is likely that this gene locus is not in the common pathway of the two diseases, resulting in the difference in results. Similarly, Xiang Q et al. also suggested that rs12569232 of TRAF5 was significantly associated with uveitis [16], while our study showed that TRAF5 rs12569232 was not significantly associated with susceptibility to AS. The reason for this inconsistency may be that the immune mechanism of TRAF5 in the two diseases is different, or it may be that the sample size of our study is insufficient, leading to different results.

Routine statistical analysis using the MDR method found that the interaction between TRAF2 and TRAF5 genes was significantly associated with ankylosing spondylitis. The optimal gene-gene interaction model was identified as three locus model, namely TRAF2 (rs10781522, rs17250673) and TRAF5 (rs4951523). Based on these findings, we speculated that because ankylosing spondylitis is a complex autoimmune disease, individual genetic mutation may only have a small edge effect on its pathogenesis, and it is difficult to detect [26]. In other words, certain components in the development of AS, such as TRAF2 and TRAF5, may act synergistically in ways that we are still unclear. TRAF2 and TRAF5 are both members of the TRAF family of genes, and they work together on many pathways. For example, the NF-κB signaling pathway can still be activated after single knockout of TRAF2 or TRAF5, but it can be deactivated after double knockout of TRAF2 and TRAF5 [16]. Therefore, it is necessary to study the relationship between the interaction between TRAF2 and TRAF5 and ankylosing spondylitis.

Additionally, rs3750511 polymorphism may be associated with sleep quality in the dominant model. The reasons for the correlation are as follows: Firstly, rs3750511 is related to disease activity, and higher disease activity may affect the sleep quality of patients. Secondly, \( P \) value is 0.036, which is greater than 0.05 after correction. Positive correlation may also be statistically correlated with accidental factors. Thirdly, there are some missing values of environmental factors investigated in this study, and false positive results may also occur. In the partially absence of the data of environmental factors, we persisted in the analysis because the results of the analysis of the association between environment and genes may provide clues for further research.

There are some limitations in this study. Firstly, the sample size of this study, especially in female subgroup, is moderate, the results should be interpreted with caution, and independent, multi-center, large-scale studies are needed to validate our results. Secondly, no correlation analysis between gene polymorphism and drugs was conducted to explore whether gene polymorphism affects patients' susceptibility to drugs, which remains to be further explored.

**Conclusion**

The present research indicated that the SNPs of TRAF2 and TRAF5 does not indicate a correlation with the susceptibility of AS in Chinese Han population, but the genotype frequency of rs3750511 was associated with female AS patients after gender stratification. And rs10781522 and rs3750511 polymorphism may be associated with disease activity in AS. Our results also showed that there were significant correlations between TRAF2 (rs10781522, rs17250673) and TRAF5 (rs4951523) and AS in gene-gene interaction model.

**Declarations**

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Conflict of interest

All authors declare they have no conflicts of interest.

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Tables

Table 1 Demographic and clinical characteristics of subjects

|                      | AS (n = 673) | HC (n = 687) | c²/t | P value |
|----------------------|--------------|--------------|------|---------|
| Age (year)           | 28.58 ± 9.31 | 28.62 ± 7.76 | 0.087| 0.931   |
| Gender (male/female) | 548/125      | 560/127      | 0.002| 0.967   |
| BMI (kg/m²)          | 22.27 ± 4.17 | -            | -    |         |
| HLA-B27(+, %)        | 413 (61.92%) | -            | -    |         |
| Disease duration (year) | 7.92 (5.92-11.92) | - |         |
| ESR (mm/h)           | 15.00 (6.00-33.00) | - |         |
| CRP (mg/L)           | 9.89 (2.81-29.82) | - |         |
| BASFI (cm)           | 0.90 (0.00-2.60) | - |         |
| BASDAI (cm)          | 2.00 (0.60-3.80) | - |         |
| ASDAS-CRP (cm)       | 3.28 (2.58-4.00) | - |         |

AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HC, Health control; -: not available.

*: normal variables were presented as means ± standard deviation (SD); b*: skewed data were expressed as median (interquartile range).

Table 2 Genotype and allelic frequencies of TRAF2 and TRAF5 in AS patients and healthy controls
| SNPs         | All subjects (n = 1360) | Female subjects (n = 252) | Male subjects (n = 1108) |
|--------------|-------------------------|---------------------------|--------------------------|
|              | AS  | HC  | c²  | P   | AS  | HC  | c²  | P   | AS  | HC  | c²  | P   |
| rs10781522   | AA  | 356 | 366 | 0.103 | 0.950 | 69  | 64  | 0.858 | 0.651 | 287 | 302 | 0.271 | 0.872 |
|              | GA  | 269 | 275 | 47   | 55   | 222 | 220 |
|              | GG  | 48  | 46  | 9    | 8    | 39  | 38  |
|              | A   | 981 | 1007| 0.057 | 0.811 | 185 | 183 | 0.224 | 0.621 | 796 | 824 | 0.251 | 0.616 |
|              | G   | 365 | 367 | 65   | 71   | 300 | 296 |
| rs12569232   | CC  | 3   | 2   | 0.447 | 0.801 | 0   | 0   | 0.270 | 0.603 | 3   | 2   | 0.270 | 0.874 |
|              | GC  | 49  | 54  | 6    | 8    | 43  | 46  |
|              | GG  | 621 | 631 | 119  | 119  | 502 | 512 |
|              | C   | 55  | 58  | 6    | 8    | 49  | 50  | <0.001 | 0.994 |
|              | G   | 1291| 1316| 244  | 246  | 1047| 1070|
| rs17250673   | AA  | 16  | 15  | 2.035 | 0.361 | 1   | 3   | 1.303 | 0.521 | 15  | 12  | 2.078 | 0.354 |
|              | GA  | 169 | 196 | 33   | 37   | 136 | 159 |
|              | GG  | 488 | 476 | 91   | 87   | 397 | 389 |
|              | A   | 201 | 226 | 35   | 43   | 166 | 183 | 0.594 | 0.441 |
|              | G   | 1145| 1148| 215  | 211  | 930 | 937 |
| rs3750511    | AA  | 17  | 9   | 3.410 | 0.208 | 3   | 0   | 5.907 | 0.033 | 14  | 9   | 2.281 | 0.216 |
|              | CA  | 153 | 169 | 27   | 41   | 126 | 128 |
|              | CC  | 502 | 509 | 95   | 86   | 407 | 423 |
|              | A   | 187 | 187 | 33   | 41   | 154 | 146 | 0.512 | 0.474 |
|              | C   | 1157| 1187| 217  | 213  | 940 | 974 |
| rs4951523    | GG  | 321 | 309 | 1.603 | 0.449 | 64  | 68  | 0.450 | 0.798 | 257 | 241 | 2.020 | 0.364 |
|              | GT  | 290 | 303 | 51   | 47   | 239 | 256 |
|              | TT  | 62  | 75  | 10   | 12   | 52  | 63  |
|              | G   | 932 | 921 | 1.532 | 0.216 | 179 | 183 | 0.012 | 0.911 | 753 | 738 | 1.989 | 0.158 |
|              | T   | 414 | 453 | 71   | 71   | 343 | 382 |
| rs59471504   | AA  | 6   | 5   | 0.948 | 0.784 | 2   | 1   | 0.779 | 0.673 | 4   | 4   | 0.234 | 0.922 |
|              | GA  | 146 | 140 | 24   | 21   | 122 | 119 |
|              | GG  | 521 | 542 | 99   | 105  | 422 | 437 |
|              | A   | 158 | 150 | 0.457 | 0.499 | 28  | 23  | 0.673 | 0.425 | 130 | 127 | 0.147 | 0.701 |
|              | G   | 1188| 1124| 222  | 231  | 966 | 993 |
| rs6540679    | AA  | 35  | 44  | 0.953 | 0.621 | 6   | 8   | 0.925 | 0.630 | 29  | 36  | 1.040 | 0.594 |
|              | GA  | 254 | 260 | 46   | 40   | 208 | 220 |
|              | GG  | 384 | 383 | 73   | 79   | 311 | 304 |
|              | A   | 324 | 348 | 0.577 | 0.448 | 58  | 56  | 0.096 | 0.757 | 266 | 292 | 0.954 | 0.329 |
|              | G   | 1022| 1026| 192  | 198  | 830 | 828 |
| rs7514863    | AA  | 629 | 628 | 2.705 | 0.199 | 117 | 117 | 1.004 | 1.000 | 512 | 511 | 1.859 | 0.713 |
AT  44  58  8  9  36  49
TT  0  1  0  1  0  1
A  1302  1314  2.229  0.135  242  243  0.444  0.505  1060  1071  1.785  0.182
T  44  60  8  11  36  49

AS, Ankylosing spondylitis; HC, Health control; P, P value; SNP, single nucleotide polymorphism; TRAF2, TNF receptor-associated factor 2; TRAF5, TNF receptor-associated factor 5.

*P* values with bold were considered statistically significant differences.

**Table 3 Genotype and allelic frequencies of TRAF2 and TRAF5 in AS patients between different clinical characteristics**
| SNP         | <4  | ≥4  | c²  | P   | <4  | ≥4  | c²  | P   | negative | positive | c²   | P   |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|--------|----------|------|-----|
| rs10781522  | AA  | 269 | 87  | 5.075 | 0.079 | 291 | 65  | 4.512 | 0.105 | 132     | 223       | 0.990 | 0.610 |
|             | GA  | 222 | 47  |       |       | 226 | 43  |       |       | 101     | 164       |      |      |
|             | GG  | 40  | 8   |       |       | 45  | 3   |       |       | 21      | 26        |      |      |
|             | A   | 760 | 221 | 4.434 | 0.035 | 808 | 173 | 3.424 | 0.064 | 365     | 610       | 0.639 | 0.424 |
|             | G   | 302 | 63  |       |       | 316 | 49  |       |       | 143     | 216       |      |      |
| rs12569232  | CC  | 3   | 0   | 1.151 | 0.562 | 2   | 1   | 3.121 | 0.210 | 2       | 1         | 1.049 | 0.592 |
|             | GC  | 37  | 12  |       |       | 37  | 12  |       |       | 18      | 30        |      |      |
|             | GG  | 491 | 130 |       |       | 523 | 98  |       |       | 234     | 382       |      |      |
|             | C   | 41  | 12  | 0.018 | 0.894 | 41  | 12  | 3.343 | 0.067 | 22      | 32        | 0.169 | 0.681 |
|             | G   | 1019 | 272 |       |       | 1083 | 208 |       |       | 486     | 794       |      |      |
| rs17250673  | AA  | 11  | 5   | 1.088 | 0.580 | 13  | 3   | 0.244 | 0.885 | 4       | 12        | 2.530 | 0.282 |
|             | GA  | 135 | 34  |       |       | 143 | 26  |       |       | 70      | 96        |      |      |
|             | GG  | 385 | 103 |       |       | 406 | 82  |       |       | 180     | 305       |      |      |
|             | A   | 157 | 44  | 0.089 | 0.776 | 169 | 32  | 0.056 | 0.812 | 78      | 120       | 0.170 | 0.680 |
|             | G   | 905 | 240 |       |       | 955 | 190 |       |       | 430     | 706       |      |      |
| rs3750511   | AA  | 15  | 2   | 10.962 | 0.004 | 15  | 2   | 2.130 | 0.345 | 7       | 10        | 0.203 | 0.904 |
|             | CA  | 127 | 26  |       |       | 133 | 20  |       |       | 59      | 91        |      |      |
|             | CC  | 388 | 114 |       |       | 413 | 89  |       |       | 188     | 311       |      |      |
|             | A   | 157 | 30  | 3.374 | 0.066 | 163 | 24  | 2.138 | 0.144 | 73      | 111       | 0.213 | 0.644 |
|             | C   | 903 | 254 |       |       | 959 | 198 |       |       | 435     | 713       |      |      |
| rs4951523   | GG  | 252 | 69  | 1.603 | 0.449 | 270 | 51  | 0.190 | 0.909 | 123     | 196       | 0.208 | 0.901 |
|             | GT  | 226 | 64  |       |       | 241 | 49  |       |       | 109     | 177       |      |      |
|             | TT  | 53  | 9   |       |       | 51  | 11  |       |       | 22      | 40        |      |      |
|             | G   | 730 | 202 | 0.600 | 0.438 | 781 | 151 | 0.187 | 0.665 | 355     | 569       | 0.146 | 0.702 |
|             | T   | 332 | 82  |       |       | 343 | 71  |       |       | 153     | 257       |      |      |
| rs59471504  | AA  | 5   | 1   | 1.882 | 0.390 | 6   | 0   | 1.532 | 0.465 | 3       | 2         | 1.180 | 0.554 |
|             | GA  | 121 | 25  |       |       | 124 | 22  |       |       | 57      | 88        |      |      |
|             | GG  | 405 | 116 |       |       | 432 | 89  |       |       | 194     | 323       |      |      |
|             | A   | 131 | 27  | 1.730 | 0.188 | 136 | 23  | 0.858 | 0.354 | 63      | 92        | 0.489 | 0.484 |
|             | G   | 931 | 257 |       |       | 988 | 200 |       |       | 445     | 734       |      |      |
| rs6540679   | AA  | 29  | 6   | 1.022 | 0.600 | 28  | 7   | 0.604 | 0.739 | 10      | 25        | 2.152 | 0.708 |
|             | GA  | 204 | 50  |       |       | 215 | 39  |       |       | 96      | 155       |      |      |
|             | GG  | 298 | 86  |       |       | 319 | 65  |       |       | 148     | 233       |      |      |
|             | A   | 262 | 62  | 0.988 | 0.320 | 271 | 53  | 0.006 | 0.940 | 116     | 205       | 0.677 | 0.410 |
|             | G   | 800 | 222 |       |       | 853 | 169 |       |       | 392     | 621       |      |      |
| rs7514863   | AA  | 496 | 133 | 0.012 | 0.914 | 527 | 102 | 0.536 | 0.464 | 239     | 385       | 0.199 | 0.655 |
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; \( P \), \( P \) value; SNP, single nucleotide polymorphism; TRAF2, TNF receptor-associated factor 2; TRAF5, TNF receptor-associated factor 5.

\( P \) values with bold were considered statistically significant differences.

**Table 4** The best model for predicting the occurrence of AS

| Model | Training accuracy | Testing accuracy | CVC | \( \chi^2 \) | \( P \) value |
|-------|-------------------|------------------|-----|----------|-------------|
| TRAF2 (rs17250673) | 0.5184 | 0.4902 | 6/10 | 1.766 | 0.184 |
| TRAF2 (rs17250673), TRAF5 (rs4951523) | 0.5328 | 0.4835 | 5/10 | 5.300 | 0.021 |
| TRAF2 (rs10781522, rs17250673), TRAF5 (rs12569232) | 0.5516 | 0.5267 | 10/10 | 13.435 | 0.0002 |

CVC: Cross-validation Consistency; TRAF2, TNF receptor-associated factor 2; TRAF5, TNF receptor-associated factor 5.

**Table 5** Univariate logistic regression analysis of environmental factor with rs3750511
| Environmental factor       | CC  | CA+AA | OR (95%CI)      | P value |
|---------------------------|-----|-------|-----------------|---------|
| **Smoking**               |     |       |                 |         |
| Yes                       | 180 | 55    | 1.031 (0.485-2.191) | 0.937   |
| No                        | 322 | 115   | Reference       |         |
| **Drinking**              |     |       |                 |         |
| Yes                       | 127 | 44    | 1.066 (0.486-2.337) | 0.873   |
| No                        | 365 | 124   | Reference       |         |
| **Salt intake level**     |     |       |                 |         |
| Low                       | 55  | 26    | Reference       |         |
| Medium                    | 281 | 93    | 1.026 (0.394-2.671) | 0.958   |
| High                      | 139 | 41    | 0.580 (0.193-1.739) | 0.331   |
| **Cooking oil**           |     |       |                 |         |
| More vegetable oils       | 87  | 26    | Reference       |         |
| Half vegetable oil        | 248 | 93    | 0.771 (0.336-1.771) | 0.540   |
| More animal oil           | 133 | 39    | 0.321 (0.103-1.005) | 0.051   |
| **Frequency of eating fatty meat** |     |       |                 |         |
| Hardly                    | 252 | 79    | Reference       |         |
| Occasionally              | 186 | 64    | 1.133 (0.559-2.299) | 0.730   |
| Frequently                | 33  | 13    | 3.221 (0.514-20.176) | 0.211   |
| **Frequency of drinking milk** |     |       |                 |         |
| Hardly                    | 113 | 36    | Reference       |         |
| Occasionally              | 30  | 17    | 0.952 (0.432-2.099) | 0.903   |
| Frequently                | 41  | 8     | 0.614 (0.223-1.689) | 0.344   |
| **Type of drinking water**|     |       |                 |         |
| Underground water         | 113 | 33    | Reference       |         |
| Tap water                 | 348 | 125   | 0.528 (0.221-1.262) | 0.151   |
| Mineral water             | 23  | 3     | 0.334 (0.058-1.930) | 0.220   |
| **Noise**                 |     |       |                 |         |
| Yes                       | 92  | 32    | 1.315 (0.552-3.314) | 0.536   |
| No                        | 373 | 125   | Reference       |         |
| **Sleep quality**         |     |       |                 |         |
| Poor                      | 122 | 30    | Reference       |         |
| Average                   | 213 | 87    | 2.446 (1.060-5.643) | 0.036   |
| Good                      | 160 | 47    | 1.522 (0.578-4.008) | 0.396   |
| **Damp condition of residence** |     |       |                 |         |
| Yes                       | 40  | 13    | 0.940 (0.404-2.186) | 0.886   |
| No                        | 166 | 47    | Reference       |         |
| **Frequency of exercise** |     |       |                 |         |
| Frequency   | Count | Ref | OR (95% CI) | P     |
|-------------|-------|-----|-------------|-------|
| Hardly      | 124   | 36  | Reference   |       |
| Occasionally| 64    | 19  | 1.231 (0.567-2.673) | 0.599 |
| Frequently  | 29    | 8   | 1.063 (0.386-2.927) | 0.906 |

OR, Odds ratios; 95% CI, 95% confidence intervals.

*P* values with bold were considered statistically significant differences.