Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

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

Shanshan Xu
Anhui Medical University
Jiangping Kong
Anhui Medical University
Li Huang
Anhui Medical College
Huimin Xie
Anhui Medical University
Feier Wang
Anhui Medical University
Tingting Zhou
Anhui Medical University
Xiaoyi Zhang
Anhui Medical University
Lingxiang Yu
Anhui Medical University
Shengqian Xu
Anhui Medical University
Faming Pan (famingpan@ahmu.edu.cn)
Anhui Medical University https://orcid.org/0000-0003-4242-1025

Research Article

Keywords: Ankylosing spondylitis, TRAF2, TRAF5, Single nucleotide polymorphisms, gene-gene interaction

Posted Date: March 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-280696/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

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 rs3750511were statistically significant in females between patients and controls. The genotype frequencies of rs12569232 and allele frequencies of rs3750511were 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 erythematous, 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 PhaseII+III, 10 August, on NCBI B36 assembly, dpSNP b126). Tag SNPs were identified as candidate SNPs to cover polymorphisms with minimum minor allele frequency $\geqslant$ 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 (χ^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≥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≥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 [(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 ≥ 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 could 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

Acknowledgments

We are deeply grateful to all the patients and healthy controls who participated in our study. And we would like to acknowledgement the national natural science foundation of China for its financial support.

Funding

The study was sponsored by the National Natural Science Foundation of China (82073655 and 81773514) and the Scientific Research Level upgrading Project of Anhui Medical University (2020xkjT006).

Conflict of interest

All authors declare they have no conflicts of interest.

References

1. Baeten D, Baraliakos X, Braun J, et al. Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. Lancet. 2013; 382: 1705-13. https://doi.org/10.1016/s0140-6736(13)61134-4
2. Ng SC, Liao Z, Yu DT, Chan ES, Zhao L, Gu J. Epidemiology of spondyloarthritis in the People's Republic of China: review of the literature and commentary. Semin Arthritis Rheum. 2007; 37: 39-47. https://doi.org/10.1016/j.semarthrit.2007.01.003
3. Zhai J, Rong J, Li Q, Gu J. Immunogenetic study in Chinese population with ankylosing spondylitis: are there specific genes recently disclosed? Clin Dev Immunol. 2013; 2013: 419357. https://doi.org/10.1155/2013/419357
4. Boonen A. A review of work-participation, cost-of-illness and cost-effectiveness studies in ankylosing spondylitis. Nat Clin Pract Rheumatol. 2006; 2: 546-53. https://doi.org/10.1038/ncprheum0297
5. Miao J, Chen Y, Zhang B, et al. Surgical Treatment for Odontoid Fractures in Patients with Long-Standing Ankylosing Spondylitis: A Report of 3 Cases and Review of the Literature. World Neurosurg. 2018; 116: 88-93. https://doi.org/10.1016/j.wneu.2018.05.055
6. Coksevim NH, Durmus D, Kuru O. Effects of global postural reeducation exercise and anti-TNF treatments on disease activity, function, fatigue, mobility, sleep quality and depression in patients with active Ankylosing spondylitis: A prospective follow-up study. J Back Musculoskelet Rehabil. 2018; 31: 1005-12. https://doi.org/10.3233/bmr-170901
7. Skuhala T, Atelj A, Prepolec J, Al-Mufleh M, Stanimirović A, Vukelić D. A case report of severe recurrent varicella in an ankylosing spondylitis patient treated with adalimumab - a new side effect after 15 years of usage. BMC Infect Dis. 2019; 19: 127. https://doi.org/10.1186/s12879-019-3768-y
8. Khan MA, Mathieu A, Sorrentino R, Akkoc N. The pathogenetic role of HLA-B27 and its subtypes. Autoimmun Rev. 2007; 6: 183-9. https://doi.org/10.1016/j.autrev.2006.11.003
9. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. Lancet. 1973; 1: 904-7. https://doi.org/10.1016/s0140-6736(73)91360-3
10. Khan MA. HLA-B27 and its subtypes in world populations. Curr Opin Rheumatol. 1995; 7: 263-9. https://doi.org/10.1097/00002281-199507000-00001
11. Hanson A, Brown MA. Genetics and the Causes of Ankylosing Spondylitis. Rheum Dis Clin North Am. 2017; 43: 401-14. https://doi.org/10.1016/j.rdc.2017.04.006
12. Uygunoğlu U, Uluduz D, Taşçılar K, Saip S. Multiple sclerosis during adalimumab treatment in a case with ankylosing spondylitis. Rheumatol Int. 2014; 34: 141-3. https://doi.org/10.1007/s00296-012-2625-8
13. Wang NG, Wang DC, Tan BY, Wang F, Yuan ZN. TNF-α and IL10 polymorphisms interaction increases the risk of ankylosing spondylitis in Chinese Han population. Int J Clin Exp Pathol. 2015; 8: 15204-9.
14. Potter C, Eyre S, Cope A, Worthington J, Barton A. Investigation of association between the TRAF family genes and RA susceptibility. Ann Rheum Dis. 2007; 66: 1322-6. https://doi.org/10.1136/ard.2006.065706
15. Qian BP, Wang XQ, Qiu Y, Jiang J, Ji ML, Feng F. Association of receptor activator of nuclear factor-kappaB ligand (RANKL) gene polymorphisms with the susceptibility to ankylosing spondylitis: a case-control study. J Orthop Sci. 2014; 19: 207-12. https://doi.org/10.1007/s00776-013-0528-5
16. Xiang Q, Chen L, Fang J, et al. TNF receptor-associated factor 5 gene confers genetic predisposition to acute anterior uveitis and pediatric uveitis. Arthritis Res Ther. 2013; 15: R113. https://doi.org/10.1186/ar4293
17. Wang X, Yang J, Han L, et al. TRAF5-mediated Lys-63-linked Polyubiquitination Plays an Essential Role in Positive Regulation of RORgammat in Promoting IL-17A Expression. J Biol Chem. 2015; 290: 29086-94. https://doi.org/10.1074/jbc.M115.664573
18. Liu J, Qi Y, Zheng L, et al. Xinfeng capsule improves pulmonary function in ankylosing spondylitis patients via NF-KappaB-iNOS-NO signaling pathway. J Tradit Chin Med. 2014; 34: 657-65.
19. Hong X, Hao K, Ladd-Acosta C, et al. Genome-wide association study identifies peanut allergy-specific loci and evidence of epigenetic mediation in US children. Nat Commun. 2015; 6: 6304. https://doi.org/10.1038/ncomms7304
20. Edmunds L, Elswood J, Calin A. New light on uveitis in ankylosing spondylitis. J Rheumatol. 1991; 18: 50-2.
21. Linssen A, Rothova A, Valkenburg HA, et al. The lifetime cumulative incidence of acute anterior uveitis in a normal population and its relation to ankylosing spondylitis and histocompatibility antigen HLA-B27. Invest Ophthalmol Vis Sci. 1991; 32: 2568-78.

22. Zeboulon N, Dougados M, Gossec L. Prevalence and characteristics of uveitis in the spondyloarthropathies: a systematic literature review. Ann Rheum Dis. 2008; 67: 955-9. https://doi.org/10.1136/ard.2007.075754
23. Ritchie MD, Hahn LW, Roodi N, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet. 2001; 69: 138-47. https://doi.org/10.1086/321276
24. Dervieux T, Wessels JA, Kremer JM, et al. Patterns of interaction between genetic and nongenetic attributes and methotrexate efficacy in rheumatoid arthritis. Pharmacogenet Genomics. 2012; 22: 1-9. https://doi.org/10.1097/FPC.0b013e32834d3e0b
25. van den Berg R, de Hooge M, van Gaalen F, Reijnierse M, Huizinga T, van der Heijde D. Percentage of patients with spondyloarthritis in patients referred because of chronic back pain and performance of classification criteria: experience from the Spondyloarthritis Caught Early (SPACE) cohort. Rheumatology (Oxford). 2013; 52: 1492-9. https://doi.org/10.1093/rheumatology/ket164
26. Lin Z, Su Y, Zhang C, et al. The interaction of BDNF and NTRK2 gene increases the susceptibility of paranoid schizophrenia. PLoS One. 2013; 8: e74264. https://doi.org/10.1371/journal.pone.0074264

Tables

Table 1 Demographic and clinical characteristics of subjects

	AS (n = 673)	HC (n = 687)	c^2/t	*P* value
Age (year)[a]	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/m^2) [a]	22.27 ± 4.17	-		
HLA-B27(+, %)	413 (61.92%)	-		
Disease duration (year)	7.92 (5.92-11.92)	-		
ESR (mm/h) [b]	15.00 (6.00-33.00)	-		
CRP (mg/L) [b]	9.89 (2.81-29.82)	-		
BASFI (cm) [b]	0.90 (0.00-2.60)	-		
BASDAI (cm) [b]	2.00 (0.60-3.80)	-		
ASDAS-CRP (cm) [b]	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.

[a]: 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

		All subjects (n = 1360)				Female subjects (n = 252)				Male subjects (n = 1108)			
SNPs		AS	HC	χ^2	*P*	AS	HC	χ^2	*P*	AS	HC	χ^2	*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	0.031	0.861	6	8	0.262	0.609	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	1.180	0.277	35	43	0.826	0.363	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	0.053	0.818	33	41	0.870	0.351	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	0448	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

		BASDAI (n=673)				BASFI (n=673)				HLA-B27 (n=667)			
SNP		<4	≥4	χ^2	*P*	<4	≥4	χ^2	*P*	negative	positive	χ^2	*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

	AT	35	9			35	9			15	28		
	TT	0	0			0	0			0	0		
	A	1027	275	0.011	0.915	1089	213	0.518	0.472	493	798	0.193	0.661
	T	35	9			35	9			15	28		

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	c^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				

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.

Figures

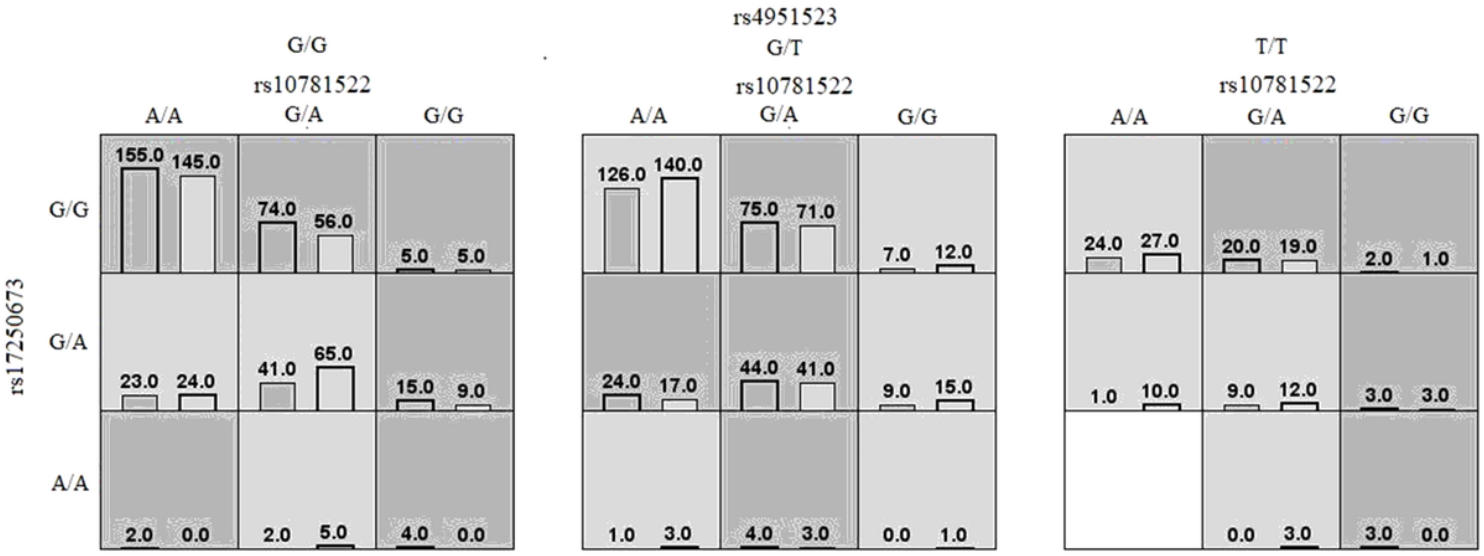

Figure 1

Distribution of high-risk and low-risk genotypes in the best three-locus model. The dark gray and light gray boxes represent high-risk and low-risk factor combinations, respectively. The left column in each box represents the AS case group, while the right column represents the control group. The height of the column is proportional to the sum of each set of samples. The different patterns of high-risk and low-risk cells across different multilocus dimensions provide evidence for gene-gene interactions. That is, the effect of each genotype of a particular locus on disease risk depends on the genotypes of the other two loci.