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The association of *NLRP3* and *TNFRSF1A* polymorphisms with risk of ankylosing spondylitis and treatment efficacy of etanercept

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Background: To discover how *NLRP3* and *TNFRSF1A* polymorphisms affect the efficacy of traditional medicine and etanercept for ankylosing spondylitis (AS) patients.

Methods: Single nucleotide polymorphism (SNP) and haplotype analyses were conducted based on determined *NLRP3* and *TNFRSF1A* among AS patients. We subsequently analyzed the relationship between relevant clinical indexes and polymorphisms of *NLRP3* and *TNFRSF1A*.

Results: The 4 SNP loci on *NLRP3* and 3 SNP loci on *TNFRSF1A* showed significant linkage disequilibrium, respectively. The T allele of *NLRP3* rs4612666 and the T allele of *TFRSF1A* rs4149570 are both associated with AS ($P<.05$). The T-A-C-T haplotype of NLRP3 as well as the G-C-C, T-C-C, T-C-T, and T-T-T haplotypes of TFRSF1A are associated with AS ($P<.05$). The morning stiffness time, BASDAI scoring, and ESR of patients receiving etanercept were significantly higher than those receiving traditional medicine. T allele of *NLRP3* rs4612666 had a significantly greater negative impact on the ASAS20 improvement than C allele. Whereas the A allele of *NLRP3* rs3806268 had a significantly greater positive impact on the ASAS20 improvement than G allele. There is no significant association between SNP and efficacy of traditional medicine in the treatment of AS.

Conclusion: *NLRP3* and *TFRSF1A* (rs4149570) are associated with AS susceptibility. There is a significant association between *NLRP3* polymorphisms and treatment of etanercept.

KEYWORDS
ankylosing spondylitis, etanercept, NLRP3, single nucleotide polymorphism, TNFRSF1A

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory arthritic condition that mainly affects the axial skeleton, peripheral joints and extra-articular manifestations (e.g., uveitis, psoriasis, inflammatory bowel disease, and cardiomyopathy).[1,2] The disease usually begins in the third decade of life and the ratio of male to female prevalence is 2:1.[3] In light of population surveys, the overall estimated AS prevalence rate is about 0.24% in Europe, 0.17% in Asia, 0.32% in North America, 0.10% in Latin America, and 0.07% in Africa.[4] The pathogenesis of AS is not completely understood. Evidence suggests that genetic factors, such as HLA B27, have a strong correlation to AS.[3] Although many studies have proved that the histocompatibility antigen, human leukocyte antigen B27 (HLA-B27), is associated with the incidence of AS, HLA-B27 only comprised one-third of the genetic components.[5]

Over the last decade, improvements in single nucleotide polymorphisms (SNPs) high-throughput genotyping and dissection of the true polygenic nature of AS have been very rapid. To date, more than 40 genetic variants and over 36 genetic loci have been identified to be associated with AS.[6] It is widely recognized that AS is a chronic

inflammatory disease and that inflammasomes of NLR family pyrin domain containing 3 (NLRP3) play a crucial role in inflammatory diseases.[7] Recently, Zhang et al.[8] demonstrated that *NLRP3* polymorphism is associated with increased susceptibility to rheumatoid arthritis in humans. Furthermore, Sode et al.[9] reported that patients with *NLRP3* (rs10754558) variant allele carriers and rheumatoid arthritis are more likely to have a negative response to TNF inhibitor treatment. The *TNFRSF1A* encodes tumor necrosis factor receptor 1 (TNF-R1) and mutations in the gene can cause autoinflammatory disorders.[10] It has been published that the *TNFRSF1A* variant is associated with AS risk (with an odds ratio of 1.1) and that TNF inhibitors are a highly effective method of treatment.[11]

Non-steroidal anti-inflammatory drugs (NSAIDs) and non-biological disease-modifying antirheumatic drugs (e.g., sulfasalazine) are mainstream pharmacologic therapies for AS.[12,13] However, many patients with AS have ongoing symptoms and they tend to develop deformities despite the use of NSAID or DMARDs. Since 2000, the use of TNF inhibitors (e.g., etanercept, infliximab, golimumab, and adalimumab) has shown rapid and sustained reductions in all clinical and laboratory measures of disease activity.[14,15] The use of TNF inhibitors is strongly recommended for patients whose clinical symptoms are not controlled by NSAIDs or DMARDs therapy, or for those whom cannot accept the adverse effects of NSAIDs or DMARDs. These agents remarkably improve both objective and subjective indicators of disease activities and functions. This includes spinal mobility, spinal stiffness, partial remission (defined as a value of two or less on a scale from 0 to 10 in each of the four domains of the ASAS 20), the bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the bath ankylosing spondylitis function index (BASFI), X-rays of the spine and the level of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[16]

In this study, we chose etanercept as the TNF inhibitor therapy for its generally recognized efficiency in AS. Few studies have investigated the association of *NLRP3* or *TNFRSF1A* with AS risk, or effects of *NLRP3* or *TNFRSF1A* on the treatment efficiency of etanercept or sulfasalazine. Therefore, there are three primary aims of this study: (1) to determine the correlation between AS and the polymorphisms or haplotypes of *NLRP3* and *TNFRSF1A*; (2) to compare the efficiency and safety of etanercept and sulfasalazine used in patients with AS; and (3) to investigate whether the polymorphisms or haplotypes of *NLRP3* and *TNFRSF1A* affect the efficacy of etanercept or sulfasalazine. All in all, we aim to further clarify the function of genetic factors in AS and to promote the development of effective diagnosis or therapeutic strategies for AS.

2 | MATERIAL AND METHODS

2.1 | Patients and study design

A total of 200 patients with AS (155 males and 45 females) were recruited from the Department of Rheumatism and Immunology in our hospital from Jan 2014 to Jan 2017. According to the random number table, patients were divided into an etanercept treatment group (78 males and 22 females) averagely aged 46.3±6.8 years old (mean course of disease 23.9±6.8 years), and a traditional drug treatment group (77 males and 23 females) averagely aged 45.4±6.7 years old (mean course of disease 23.0±6.7 years) (Table S1). There was no significant difference in gender, age and course of disease between the experimental group and the control group ($P>.05$). Another group of 200 normal healthy individuals was collected over the same time period to be the healthy control group. This group will also participate in physical examinations and was made up of 104 males and 96 females with an average age of 45.7±8.3 years old.

2.2 | Inclusion and exclusion criteria

Inclusion criteria: (1) Diagnosed with AS according to the revised standard of New York in 1984; (2) Not being treated with corticosteroids or other immunosuppressive drugs; (3) Have normal hepatic and renal function; (4) Participants are not allergic to antibiotics such as sulfanilamide *etc*.; (5) Signed informed consent.

Exclusion criteria: (1) Have cardiac insufficiency, tuberculosis infection, active HBV or other acute infectious diseases; (2) Have peptic ulcer, chronic nephritis, diabetes, chronic obstructive pneumonia, or other chronic diseases.

2.3 | Treatment

All the patients accepted the same non-steroidal anti-inflammatory drugs (NSAIDs) as a basic treatment. They were allowed to eat 200 mg celecoxib capsules or 7.5 mg meloxicam tablets twice a day. Patients in the traditional group underwent the basic treatment and pyridine nitrogen treatment (0.75 g, three times a day, oral). The NSAIDs were discontinued after 4 weeks of disease control while the pyridine nitrogen tablets remained in use for a total of 3 months. The etanercept group was treated with the basic and etanercept treatment (25 mg, twice a week, subcutaneous injection in the upper arm). The NSAIDs were discontinued after 4 weeks of disease control while etanercept was continued and used for a total of 3 months.

2.4 | Genotyping

DNA samples from the control and AS group were isolated using the Oragene™ DNA Self-Collection kit (DNA Genotek Inc., Ottawa, Canada). Real-time fluorescent quantitative PCR (FQ-PCR) was used to detect the SNP polymorphisms of *NLRP3* and *TNFRSF1A*. About 5 mL of blood was collected from each person and mixed with 0.4 mL 15 g/L ethylenediaminetetraacetic acid-Na_2 (EDTA-Na_2) for anticoagulation. Samples were digested using proteinase K, maintained at −80°C and DNA was extracted in accordance to the QIAamp DNAKit (Qiagen, Hilden, Germany). In the NCBI SNP database, the base sequences of NLRP3 (rs4612666, rs3806268, rs10925019, rs3806265) and TNFRSF1A (rs4149570, rs767455, rs4149621, rs4149569) were found and intercepted for the DNA sequence containing SNP. We used Primer 3.0 and PrimerExpress1.5 to design primers. The length of the primer was approximately 40 bp and the ratio of GC in the primer

TABLE 1 Primer sequences of *NLRP3* and *TNFRSF1A*

Gene	SNP	Primer
NLRP3	rs4612666	F: 5′-TGCTTAAGGCCATTAATTGTG-3′
		R: 5′-CTCCACCATGGACAAGGAAG-3′
	rs3806268	F: 5′-GGATTGGGAAAACAATCCTGGC-3′
		R: 5′-CTGTCTTGGTAGAGTGTCCCC-3′
	rs10925019	F: 5′-GGAGACTGGTTGTTTGGGACA-3′
		R: 5′-TGGCAGTGGGGAGAGAATTT-3′
	rs3806265	F: 5′-GGACAGTGGGAACACATGCT-3′
		R: 5′-GGGAGCATTTCTGCACTCCTA-3′
TNFRSF1A	rs4149570	F: 5′-TCTCAGACACATAACTGAAACTGT-3′
		R: 5′-CCAGGAGACAGGTTATCTCCAC-3′
	rs767455	F: 5′-TAGCTGTCTGGCATGGGCCTCT-3′
		R: 5′-CCTACTCCAAAAGGCGGATGAA-3′
	rs4149569	F: 5′-TCTCTCATAGCCAAAGGGGC-3′
		R: 5′-TCCAGAAACCCAATGTGCCA-3′
	rs4149621	F: 5′-TTTTAGCTAAGAATGTGTCTTGGAC-3′
		R: 5′-TTGGAAAACAGATCCAGACAGT-3′

F, Forward primer; R, Reverse primer; SNP, single nucleotide polymorphism.

was 50–55%. The T_m value was roughly 60°C. PCR primer information is shown in Table 1. PCR primers and probes technology was used by Genomics for genotyping. Specifically, real-time quantitative polymerase chain reaction (RT-PCR) was conducted to determine genotypes. The reaction system contained 1–5 ng of dried genomic DNA, 2.5 μL Taqman universal PCR master mix (2×), 0.25 μL 40× SNP genotyping assay, 1.25 μL ddH_2O and 1 μL DNA template. All the amplification process was performed on ABI 7900 real-time PCR amplification instrument. The reaction condition was mainly summarized as 40 cycles of 95°C for 10 minutes, 92°C for 15 seconds, and 60°C for 1 minute. Finally, the results were determined strictly according to instructions of TaqMan® Universal PCR Master Mix kit.[17]

2.5 | Evaluating indicators

In order to analyze the different effects of etanercept and traditional medicine in the treatment of AS, a comprehensive evaluation of the clinical indicators was measured before and after treatment. The clinical indicators included were: morning waist numb time, visual analog scale (VAS), BASDAI, BASFI, ESR, and CRP. In order to analyze different genotypes in patients with different efficacies of etanercept and traditional therapy, the rate of ASAS20 improvement was calculated after 12 weeks of treatment. The ASAS20 improvement criteria[18] were as follows: (1) a >20% improvement and absolute increase of ≥1 unit of VAS, BASFI, or BASDAI scores in at least three of the four

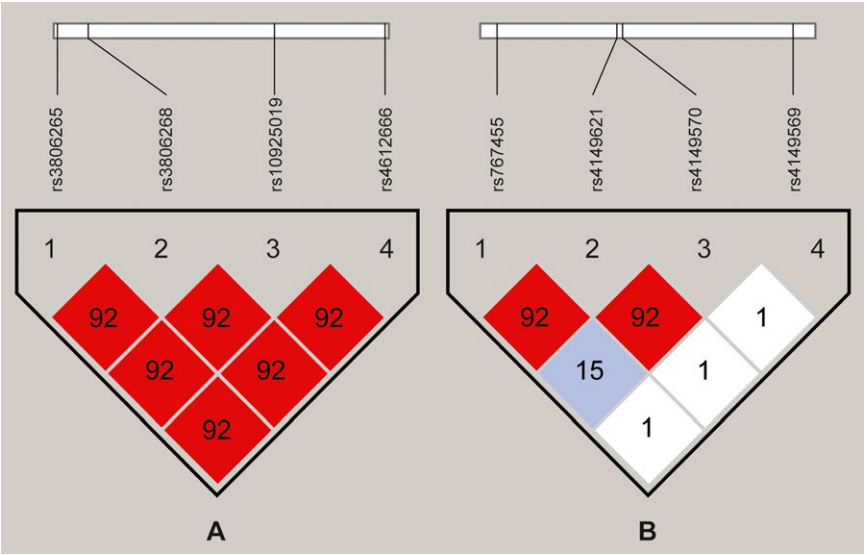

FIGURE 1 (A) Linkage disequilibrium analysis of NLRP3 (rs4612666 - rs3806265 - rs3806268 - rs10925019). (B) Linkage disequilibrium analysis of TNFRSF1A (rs4149570 - rs767455 - rs4149569 - rs4149621)

indicators overall evaluation of patient; and (2) no aggravation in the indicator that did not achieve 20% improvement.

2.6 | Statistical analysis

Statistical analysis of the linkage disequilibrium between different loci of *NLRP3* and *TNFR1A* genotypes was performed using HaploView. SPSS21.0 was used to perform the corresponding data analysis. Measurement data were displayed as mean±standard deviation (SD). The comparison between the two groups was completed using *t* test and the paired *t* test was used for data with a normal distribution. If the data were not distributed normally, the rank sum test (Mann-Whitney assay) was applied. Single factor analysis of variance (ANOVA One-way) or the non-parametric Kruskal-Wallis test was used to compare the measurement data between groups. Comparison of counting data was performed by using the Chi-square test. Logistic regression analysis was used to analyze the related factors that influence occurrence of the disease. $P<.05$ was considered statistically significance.

3 | RESULTS

3.1 | Linkage disequilibrium analysis

The r^2, SNP of *NLRP3*, and *TNFRSF1A* were analyzed using HaploView software. The results of the linkage disequilibrium analysis are indicated in the r^2 black-white graph. Black blocks represent high linkage disequilibrium (r^2=.8-1) and white blocks represent low linkage disequilibrium. As shown in Figure 1A, there is a linkage disequilibrium among the 4 SNPs of *NLRP3* (rs4612666 - rs3806265 - rs3806268 - rs10925019). Figure 1B also shows a linkage disequilibrium among the 3 SNPs of *TNFRSF1A* (rs4149570 - rs767455 - rs4149569). However, no linkage disequilibrium is seen between *TNFRSF1A* (rs4149621) and any other SNP (Figure 1B).

3.2 | Association of *NLRP3* and *TFRSF1A* polymorphisms with AS susceptibility

The loci of *NLRP3* and *TFRSF1A* SNPs are the independent variables and the contraction of AS is the dependent variable. Logistic regression analysis was performed and the results are shown in Table 2. The T allele of *NLRP3* (rs4612666) is associated with AS ($P<.05$) and T allele of TFRSF1A (rs4149570) is associated with AS ($P<.05$).

3.3 | Association of *NLRP3* haplotypes with AS susceptibility

The NLRP3 haplotypes are the independent variables and the contraction of AS is the dependent variable. Logistic regression analysis was performed. The results are shown in Table 3. The T-A-C-T haplotype of NLRP3 (rs4612666, rs3806268, rs10925019, rs3806265) is associated with AS ($P<.05$). Other haplotypes did not show significant differences.

3.4 | Association of *TFRSF1A*haplotypes with AS susceptibility

The *TFRSF1A* haplotypes are the independent variables and the contraction of AS is the dependent variable. Logistic regression analysis was performed. The results are shown in Table 4. The G-C-C, T-C-C, T-C-T, and T-T-T haplotypes of *TFRSF1A* (rs4149570, rs767455, rs4149569) are associated with AS ($P<.05$). Other haplotypes did not show significant differences.

3.5 | Comparison of etanercept and traditional medicine

As shown in Figure 2 and Table 5, the clinical indicators (morning stiffness time, VAS, BASFI, BASDAI, ESR, and CRP) of both the etanercept and traditional medicine groups changed after 12 weeks of treatment ($P<.05$). Furthermore, changes of morning stiffness time, BASDAI and ESR in the etanercept group were substantially better than that of the traditional medicine group (Table 5) ($P<.05$). In regards to VAS, BASFI and CRP, there is no statistical significance ($P>.05$) between two groups.

3.6 | Association of *NLRP3* and *TFRSF1A* polymorphisms with the efficacy of etanercept or tradition treatment for AS

After 12 weeks of etanercept or traditional treatments, ASAS20 was performed to detect the association between the SNPs and both treatments. Results are shown in Table 6. The T allele of NLRP3 (rs4612666) had a greater negative impact on the rate of ASAS20 improvement than C allele ($P<.05$). The G allele of NLRP3 (rs3806268) had a positive impact on the rate of ASAS20 improvement ($P<.05$) (Table 6). As shown in Table 7, there is no statistically significant association between SNPs and traditional drug therapy for AS ($P<.05$).

3.7 | Association of *NLRP3* and *TFRSF1A* haplotypes with efficacy of etanercept or traditional drug treatment for AS

As shown in Table 8, there is no statistically significant association between *NLRP3* haplotypes and etanercept treatment ($P>.05$). Table 9 shows that there is also no statistically significant association between *TFRSF1A* haplotypes and etanercept treatment for AS ($P>.05$). Tables 10 and 11 indicate that there is no statistically significant association between *NLRP3* or *TFRSF1A* haplotypes and traditional treatment for AS ($P>.05$).

4 | DISCUSSION

AS is a common chronic inflammatory disease which primarily affects the spinal and sacroiliac joints. Common symptoms include soreness, rigidity, and advanced deterioration of involved joints.[19,20] Genes,

TABLE 2 Association of gene polymorphisms of *NLRP3* and *TFRSF1A* with susceptibility to AS

			Genotype (n)			HWE	Dominant model		Allele frequency			Allelic model	
SNP	Mutant	Population	11	12	22	*P* value	OR (95% CI)	*P* value	1	2	MAF	OR (95% CI)	*P* value
NLRP3													
rs4612666	C>T	Case (n=100)	54	100	46	.982	1.622 (1.062-2.478)	.025	208	192	0.48	1.459 (1.102-1.933)	.008*
		Control (n=100)	75	95	30	.993	Ref.		245	155	0.63	Ref.	
rs3806268	A>G	Case (n=100)	100	83	17	.970	0.961 (0.649-1.422)	.842	283	117	0.29	0.965 (0.712-1.307)	.816
		Control (n=100)	98	84	18	.867	Ref.		280	120	0.30	Ref.	
rs10925019	C>T	Case (n=100)	118	70	12	.707	1.087 (0.728-1.622)	.683	306	94	0.24	1.105 (0.793-1.540)	.554
		Control (n=100)	122	69	9	.848	Ref.		313	87	0.22	Ref.	
rs3806265	C>T	Case (n=100)	86	91	23	.885	0.960 (0.646-1.427)	.840	263	137	0.34	0.989 (0.739-1.324)	.941
		Control (n=100)	84	94	22	.572	Ref.		262	138	0.35	Ref.	
TNFRSF1A													
rs4149570	G>T	Case (n=100)	58	99	43	.950	1.632 (1.076-2.475)	.021	215	185	0.46	1.481 (1.116-1.965)	.006*
		Control (n=100)	80	93	27	.997	Ref.		253	147	0.37	Ref.	
rs767455	C>T	Case (n=100)	85	91	24	.962	0.960 (0.645-1.428)	.839	261	139	0.35	0.963 (0.720-1.289)	.800
		Control (n=100)	83	89	26	.781	Ref.		255	141	0.35	Ref.1413	
rs4149569	C>T	Case (n=100)	53	101	46	.874	1.217 (0.788-1.881)	.376	207	193	0.48	1.140 (0.863-1.505)	.360
		Control (n=100)	61	98	41	.912	Ref.		220	180	0.45	Ref.	
rs4149621	A>G	Case (n=100)	62	99	39	.963	1.000 (0.655-1.528)	1.000	223	177	0.44	1.010 (0.764-1.336)	.943
		Control (n=100)	62	100	38	.836	Ref.		224	176	0.44	Ref.	

SNP, single nucleotide polymorphism; 11, wild homozygote; 12, heterozygote; 22, mutant homozygote; HWE, Hardy-Weinberg equilibrium; Dominant model: (22+12)/11; Allelic model: 2/1; OR, odds ratio; MAF, minor allele frequency; CI, confidence interval; *P* value.

*Significant differences.

TABLE 3 Association of *NLRP3* haplotypes with susceptibility to AS

Haplotypes	AS (n=400)	Control (n=386)	AOR (95% CI)	*P* value
C-A-C-C	101	119	Ref	-
C-A-C-T	30	38	1.081 (0.562-2.079)	.816
C-A-T-C	25	21	1.653 (0.795-3.434)	.178
C-A-T-T	9	7	1.795 (0.522-6.170)	.353
C-G-C-C	28	27	1.308 (0.622-2.751)	.478
C-G-C-T	10	10	0.884 (0.250-3.131)	.849
C-G-T-C	3	5	0.272 (0.041-1.794)	.176
C-G-T-T	4	8	2.461 (0.912-6.641)	.075
T-A-C-C	46	35	0.802 (0.400-1.607)	.534
T-A-C-T	38	25	2.156 (1.125-4.129)	.021*
T-A-T-C	20	14	1.877 (0.782-4.509)	.159
T-A-T-T	14	15	1.232 (0.476-3.189)	.667
T-G-C-C	28	24	1.779 (0.876-3.615)	.111
T-G-C-T	25	22	1.548 (0.728-3.290)	.256
T-G-T-C	12	9	1.001 (0.330-3.036)	.999
T-G-T-T	7	7	1.023 (0.257-4.062)	.975

AS, ankylosing spondylitis; Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.
*Significant differences.

TABLE 4 Association of *TFRSF1A* haplotypes with susceptibility to AS

Haplotypes	AS (n=400)	Control (n=396)	AOR (95% CI)	*P* value
G-C-C	99	138	Ref	-
G-C-T	65	45	2.284 (1.319-3.957)	.003*
G-T-C	30	34	1.334 (0.665-2.677)	.417
G-T-T	21	33	1.106 (0.540-2.267)	.782
T-C-C	43	32	2.287 (1.225-4.271)	.009*
T-C-T	54	40	2.243 (1.258-3.999)	.006*
T-T-C	35	31	1.878 (0.957-3.683)	.067
T-T-T	53	43	1.918 (1.075-3.425)	.028*

AS, ankylosing spondylitis; Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.
*Significant differences.

such as *HLA-B27*, *NLRP3*, and *TFRSF1A*, have been reported to be involved in the etiology of AS. Neeraj et al. discovered that the *HLA-B27* positive phenotype is correlated with AS. Another study conducted on a Swedish population declared there is no association between *NLRP3* SNPs and AS susceptibility. However, there were still several identified SNPs predicting treatment response to the first anti-TNF-α agent in AS.[21–23] In this study, we consistently demonstrate that there are significant correlations between *NLRP3* polymorphisms, *NLRP3* haplotypes, *TFRSF1A* rs4149570, and AS susceptibility.

The NLR family comprises three proteins of NLRP1, NLRP3, and NLRC4.[24] These proteins interact with various adaptor proteins to form a macromolecular complex called inflammasome. Inflammasome induces the activation of caspase-1 and the secretion of interleukin-1β (IL-1βy.[24] NLRP3 is also known as CIASI, NALP3, PYPAF1, and cryopyrin. It can bind to adaptor proteins such as TUCAN (CARD8) and ASC to form inflammasome.[25,26] From there, it culminates to convert procaspase 1 into caspase 1, and also processes pro-interleukin-18 (proIL-18) and pro-interleukin-1b (proIL-1b), resulting in the production of active IL-18 and IL-1b.[24] IL-18 and IL-1b are both pro-inflammatory cytokines known as puissant mediators of inflammation and are associated with autoimmune disorders such as Behcet's syndrome, AIDS, Crohn's disease, and celiac disease.[24,25,27–29] *NLRP3* polymorphisms at positions rs10754558 and rs4612666 have been shown to be related to HIV infection, type 1 diabetes, food-induced anaphylaxis, and rheumatoid arthritis. Functional analysis has previously revealed that the risk alleles of rs10754558 and rs4612666 can result in increased stability of NLRP3 mRNA and enhance NLRP3 expression.[24,26,29–31] In the present study, we discovered that the rs4612666 (C/T) in patients could increase NLRP3 mRNA stability and enhance NLRP3 activity. This subsequently led to a series of inflammatory reactions which was

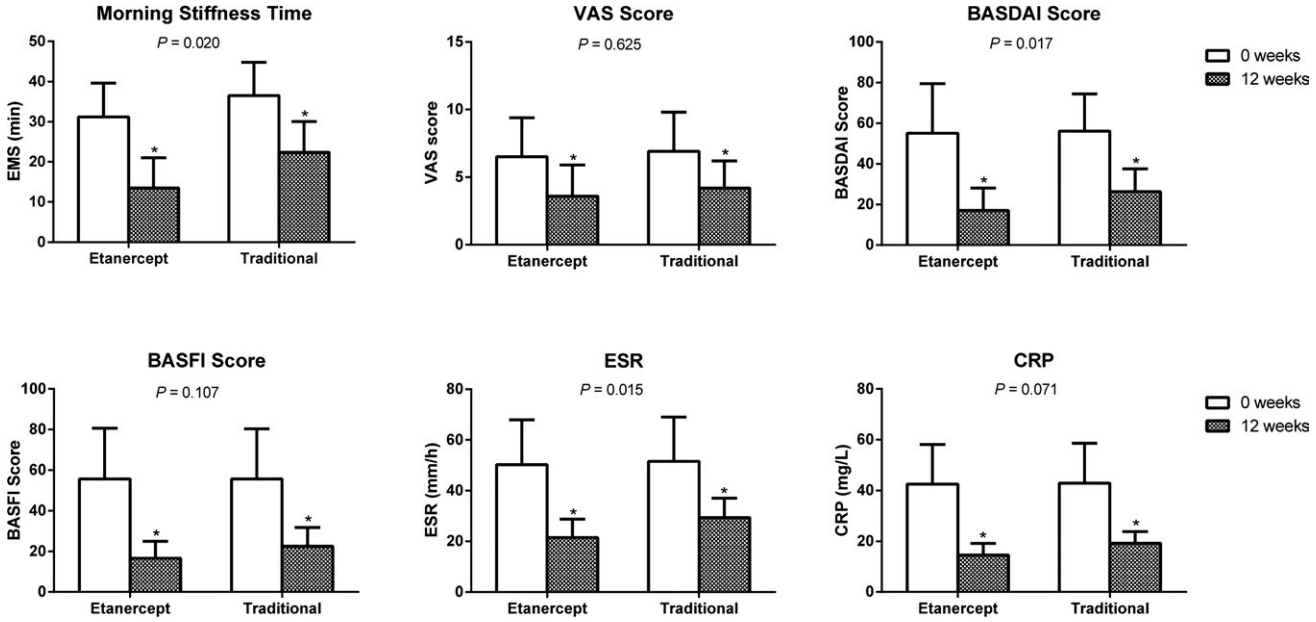

FIGURE 2 A comparison of the six clinical parameters used in the etanercept and traditional medicine groups. *Compared with previous treatment, the clinical indexes after treatments were statistically significant. $P<.05$ indicates the greater improvements in the etanercept group over the traditional medicine group are statistically significant

TABLE 5 The situation of six clinical parameters of etanercept group and traditional medicine group

Group (n=100)	Time	Morning stiffness time (minutes)	VAS	BASDAI	BASFI	ESR (mm/h)	CRP (mg/L)
Etanercept	0 weeks	31.2±8.4	6.5±2.9	55.08±24.4	55.7±24.9	50.2±17.8	42.5±15.6
	12 weeks	13.5±7.5[a]	3.6±2.3[a]	17.0±11.0[a]	16.5±8.4[a]	21.4±7.3[a]	14.5±4.6[a]
	Difference	17.7±10.7[b]	2.9±3.6	38.1±26.5[b]	39.1±26.3	28.8±18.8[b]	28.0±17.1
Traditional Medicine	0 week	36.5±8.3	6.9±2.9	56±18.44	55.6±24.7	51.5±17.5	42.9±15.8
	12 weeks	22.4±7.6[a]	4.2±2.0[a]	26.3±11.2[a]	22.6±9.2[a]	29.4±7.7[a]	19.2±4.6[a]
	Difference	14.1±11.8[b]	2.7±3.4	29.7±22.6[b]	33.1±26.3	22.1±19.5[b]	23.7±16.6

VAS, visual analog scale; BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis function index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

[a]Comparison between pre- and pro- treatmentshowed significant difference ($P<.05$).

[b]Comparison between etanercept group and traditional medicine group showed significant difference ($P<.05$).

consistent with the findings of Hitomi et al.[31] Our study also illustrated that the *NLRP3* rs3806265 in AS patients was overexpressed. We thus hypothesized that it could affect *NLRP3* synthesis and culminated to the overproduction of IL-1b (a cytokine involved in the development of AS).[32] The previous studies reported that the *NLRP3* rs10925019 was associated with Crohn's and ulcerative colitis disease. We found a close relationship between rs10925019 and AS occurrence. However, pathomechanism needed further study.[33,34]

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine which acts as the ligand for TNF-R1 and TNF-R2. It plays a vital part in the pathomechanism of rheumatoid arthritis (RA) spondyloarthritis (SpA), psoriatic arthritis (PsA), and AS.[35,36] The expression of adhesion molecules and the increase of neutrophil activation can be stimulated by TNF-α.[37] Furthermore, at a cellular level, when ligands bind to receptors it can induce apoptosis through the exterior pathway bearing cytoplasmic death domains (death receptors) such as TNF-R1 and TRAIL-R1.[37] The *TNF*-α contains several SNPs, which have been found to be relevant to susceptibility and polymorphisms.[37]

The results of our study indicate that there is linkage disequilibrium in TNFRSF1A rs4149570, rs767455 and rs4149569 of AS patients. One study reported a significant positive association between carriers of allele G and treatment response. However, our experiment illustrated that allele T in rs4149570 has a significant correlation with the incidence of AS. AS and RA are different diseases, have different mechanisms and our results may offer a crucial supplement for AS genetic diagnosis.[38] TNF blockers have been proven to be highly effective in reducing Spondyloarthritis (SpA) and inflammatory bowel diseases (IBDs).[39,40] Currently, there are a few TNF-α inhibitors available for clinical treatments. These inhibitors act to block the binding of TNF-α to its receptors and therefore, interfering with TNF-α signaling

TABLE 6 Association of gene polymorphisms of *NLRP3* and *TFRSF1A* with the efficacy of etanercept treatment for AS

Gene	SNP		Sample size (n)	ASAS20 effects		AOR (95% CI)	*P* value
				Yes (n)	No (n)		
NLRP3	rs4612666						
	Genotype	CC	26	22	4	Ref.	
		CT	47	33	14	0.429 (0.125-1.474)	.172
		TT	27	14	13	0.196 (0.053-0.723)	.011
	Allele	C	99	77	22	Ref.	
		T	101	61	40	0.436 (0.235-0.810)	.008
	rs3806268						
	Genotype	AA	61	39	22	Ref.	
		AG	23	16	7	1.289 (0.460-3.614)	.628
		GG	16	14	2	3.949 (0.820-19.010)	.070
	Allele	A	145	94	51	Ref.	
		G	55	44	11	2.170 (1.032-4.565)	.038
	rs10925019						
	Genotype	CC	62	40	22	Ref.	
		CT	28	21	7	0.606 (0.223-1.650)	.325
		TT	10	8	2	0.455 (0.089-2.331)	.335
	Allele	C	152	101	51	Ref.	
		T	48	37	11	1.698 (0.800-3.606)	.165
	rs3806265						
	Genotype	CC	54	36	18	Ref.	
		CT	27	20	7	1.429 (0.510-4.003)	.496
		TT	19	13	6	1.083 (0.353-3.323)	.889
	Allele	C	135	92	43	Ref.	
		T	65	46	19	1.132 (0.593-2.158)	.707
TNFRSF1A	rs4149570						
	Genotype	GG	34	27	7	Ref.	
		GT	43	28	15	0.484 (0.171-1.371)	.168
		TT	23	14	9	0.403 (0.124-1.313)	.126
	Allele	G	111	82	29	Ref.	
		T	89	56	33	0.600 (0.328-1.098)	.096
	rs767455						
	Genotype	CC	50	36	14	Ref.	
		CT	35	25	10	0.972 (0.373-2.536)	.954
		TT	15	8	7	0.444 (0.136-1.458)	.175
	Allele	C	135	97	38	Ref.	
		T	65	41	24	0.669 (0.357-1.254)	.209
	rs4149569						
	Genotype	CC	32	23	9	Ref.	
		CT	45	28	17	0.645 (0.242-1.715)	.377
		TT	23	18	5	1.409 (0.401-4.944)	.592
	Allele	C	109	74	35	Ref.	
		T	91	64	27	1.121 (0.613-2.050)	.710

(Continues)

TABLE 6 (Continued)

Gene	SNP		Sample size (n)	ASAS20 effects		AOR (95% CI)	*P* value
				Yes (n)	No (n)		
	rs4149621						
	Genotype	AA	34	26	8	Ref.	
		AG	43	26	17	0.471 (0.173-1.281)	.136
		GG	23	17	6	0.872 (0.257-2.961)	.826
	Allele	A	111	78	33	Ref.	
		G	89	60	29	0.875 (0.480-1.598)	.664

AS, ankylosing spondylitis; SNP, single nucleotide polymorphism; AOR, adjusted odds ratio; CI, confidence interval; ASAS20, ankylosing spondylitis assessment study 20; Ref., reference.

TABLE 7 Association of gene polymorphisms of *NLRP3* and *TFRSF1A* with the efficacy of traditional treatment for AS

Gene	SNP		Sample size (n)	ASAS20 effects		AOR (95% CI)	*P* value
				Yes (n)	No (n)		
NLRP3	rs4612666						
	Genotype	CC	27	18	9	Ref.	
		CT	51	33	18	0.917 (0.342-2.455)	.863
		TT	22	14	8	0.875 (0.269-2.851)	.825
	Allele	C	105	69	36	Ref.	
		T	95	61	34	0.936 (0.523-1.675)	.824
	rs3806268						
	Genotype	AA	52	34	18	Ref.	
		AG	34	21	13	0.855 (0.349-2.098)	.733
		GG	14	10	4	1.324 (0.363-4.822)	.670
	Allele	A	138	89	49	Ref.	
		G	62	41	21	1.075 (0.572-2.021)	.823
	rs10925019						
	Genotype	CC	66	45	21	Ref.	
		CT	22	14	8	0.817 (0.297-2.246)	.695
		TT	12	6	6	0.467 (0.134-1.620)	.223
	Allele	C	154	104	50	Ref.	
		T	46	26	20	0.625 (0.319-1.226)	.170
	rs3806265						
	Genotype	CC	45	25	20	Ref.	
		CT	38	30	8	3.000 (1.129-7.969)	.025
		TT	17	10	7	1.143 (0.369-3.542)	.817
	Allele	C	128	80	48	Ref.	
		T	72	50	22	1.364 (0.736-2.525)	.323
TNFRSF1A	rs4149570						
	Genotype	GG	29	20	9	Ref.	
		GT	46	29	17	0.768 (0.286-2.064)	.600
		TT	25	16	9	0.800 (0.257-2.487)	.700
	Allele	G	104	69	35	Ref.	
		T	96	61	35	0.884 (0.494-1.582)	.678

(Continues)

TABLE 7 (Continued)

Gene	SNP		Sample size (n)	ASAS20 effects		AOR (95% CI)	*P* value
				Yes (n)	No (n)		
	rs767455						
	Genotype	CC	42	28	14	Ref.	
		CT	42	26	16	0.813 (0.332-1.987)	.649
		TT	16	11	5	1.100 (0.319-3.789)	.880
	Allele	C	124	82	42	Ref.	
		T	76	48	28	0.878 (0.484-1.594)	.669
	rs4149569						
	Genotype	CC	28	20	8	Ref.	
		CT	42	27	15	0.720 (0.256-2.027)	.533
		TT	30	18	12	0.600 (0.200-1.800)	.360
	Allele	C	98	67	31	Ref.	
		T	102	63	39	0.747 (0.417-1.340)	.328
	rs4149621						
	Genotype	AA	35	24	11	Ref.	
		AG	42	29	13	1.022 (0.388-2.693)	.964
		GG	23	12	11	0.500 (0.169-1.481)	.208
	Allele	A	112	77	35	Ref.	
		G	88	53	35	0.688 (0.384-1.235)	.210

AS, ankylosing spondylitis; SNP, single nucleotide polymorphism; AOR, adjusted odds ratio; CI, confidence interval; ASAS20, ankylosing spondylitis assessment study 20; Ref., reference.

TABLE 8 Association between *NLRP3* haplotypes and etanercept treatment for AS

Haplotype	ASAS20 effects/total	AOR (95% CI)	*P* value
C-A-C-C	35/50	Ref	-
C-A-C-T	9/12.	1.29 (0.301-5.525)	.731
C-A-T-C	9/11	2.198 (0.417-11.596)	.353
C-A-T-T	6/6/	-	.999
C-G-C-C	14/16	2.667 (0.53-13.42)	.234
C-G-C-T	3/4	1.011 (0.093-10.991)	.993
C-G-T-C	1/2	0.279 (0.016-4.963)	.385
C-G-T-T	2/2	-	.999
T-A-C-C	14/27	0.396 (0.145-1.079)	.070
T-A-C-T	10/22	0.373 (0.129-1.08)	.069
T-A-T-C	5/10	0.391 (0.096-1.599)	.191
T-A-T-T	6/7	2.536 (0.274-23.512)	.413
T-G-C-C	9/12	1.206 (0.281-5.175)	.801
T-G-C-T	7/9	1.647 (0.3-9.056)	.566
T-G-T-C	5/7	0.947 (0.16-5.596)	.952
T-G-T-T	3/3	-	.999

AS, ankylosing spondylitis; Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.

transduction pathways, such as anti-TNF-α mAbs (golimumab, adalimumab, infliximab, and certolizumab pegol) and etanercept (a fusion protein which acts as a "decoy receptor" for TNF-α).[41–43] Etanercept is composed of two p75 TNF receptors fused with the Fc portion of human IgG1. It is generally administered subcutaneously 25 mg/d twice a week or 50 mg once a week.[44] Etanercept binds to TNF

TABLE 9 Association between TFRSF1A haplotypes and etanercept treatment for AS

Haplotype	ASAS20effects/total	AOR (95% CI)	*P* value
G-C-C	38/52	Ref	-
G-C-T	23/29	1.469 (0.482-4.483)	.499
G-T-C	12/19	0.656 (0.211-2.036)	.465
G-T-T	9/11	1.634 (0.310-8.625)	.563
T-C-C	15/24	0.681 (0.238-1.943)	.472
T-C-T	21/30	0.930 (0.339-2.545)	.887
T-T-C	9/14	0.587 (0.164-2.110)	.415
T-T-T	11/21	0.466 (0.160-1.361)	.163

AS, ankylosing spondylitis; Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.

TABLE 10 Association between *NLRP3* haplotypes and tradition treatment for AS

Haplotype	ASAS20effects/total	AOR (95% CI)	*P* value
C-A-C-C	34/51	Ref	-
C-A-C-T	12/18	0.911 (0.287-2.890)	.874
C-A-T-C	8/14	0.601 (0.176-2.047)	.415
C-A-T-T	1/3	0.215 (0.018-2.632)	.229
C-G-C-C	9/12/	1.394 (0.330-5.896)	.652
C-G-C-T	5/6	2.445 (0.259-23.056)	.435
C-G-T-C	1/1	-	1
C-G-T-T	1/2	0.521 (0.030-8.993)	.653
T-A-C-C	10/19	0.537 (0.182-1.585)	.26
T-A-C-T	12/16	1.437 (0.400-5.161)	.578
T-A-T-C	7/10	1.109 (0.252-4.874)	.891
T-A-T-T	5/7	1.054 (0.179-6.213)	.953
T-G-C-C	10/16	0.819 (0.253-2.649)	.739
T-G-C-T	12/16	1.485 (0.414-5.325)	.544
T-G-T-C	1/5	0.103 (0.010-1.034)	.053
T-G-T-T	2/4	0.523 (0.067-4.069)	.536

AS, ankylosing spondylitis; Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.

TABLE 11 Association between *TFRSF1A* haplotypes and tradition treatment for AS

Haplotype	ASAS20 effective/total	AOR (95% CI)	*P* value
G-C-C	33/47	Ref	-
G-C-T	24/36	0.868 (0.341-2.214)	.768
G-T-C	5/11	0.320 (0.081-1.263)	.104
G-T-T	7/10	0.994 (0.217-4.55)	.994
T-C-C	12/19	0.680 (0.219-2.111)	.504
T-C-T	13/24	0.468 (0.167-1.311)	.149
T-T-C	17/21	1.670 (0.470-5.932)	.428
T-T-T	19/32	0.611 (0.237-1.574)	.307

Ref, Reference level; AOR, adjusted odds ratio after age and gender correction; CI, confidence interval; *P* value.

(primarily to its soluble form) with a high affinity and blocks the effects of TNF.[42,45]

Our studies have demonstrated that there are obvious differences in the sedimentation rate (ESR) level, waist duration of morning stiffness and BASFI between traditional medicine and etanercept treatment patients. We also observed no significant differences in VAS, CRP between the two groups. We conclude that the baseline factors which are significant predictors of ASAS 20 response in etanercept-treated patients are ESR, waist duration of morning stiffness, and the BASFI score. This conclusion is partly contradictory to the findings of Davis et al.[46] When patients are treated with etanercept, we discovered that the allele T has a greater negative influence on the achievement of ASAS20 than C of rs4612666 in NLRP3. Furthermore, the allele G of rs3806268 showed a better achievement of ASAS20 than A. This indicates that the two genes may be detection factors and predictors of AS.

The major limitation of this study would be the small sample size. We intended to conduct in-depth research on a larger population for the next stage. In conclusion, we have studied the association of *NLRP3* and *TNFRSF1A* polymorphisms and haplotypes with AS susceptibility. The correlation of *NLRP3* and *TNFRSF1A* polymorphisms and haplotypes with the curative effect and adverse reactions of AS patients treated with traditional medicines and etanercept. Our results imply that *NLRP3* SNPs and haplotypes play a critical role in the development of ankylosing spondylitis. Further research on *NLRP3* inflammasome will contribute to the development of diagnostic and therapeutic methods for AS.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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