Association of Genetic Variations in *TNFSF15* With Acute Anterior Uveitis in Chinese Han

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Purpose. T cells play an important role in the pathogenesis of uveitis. Recent studies have indicated that the *TNFSF15* gene that encodes the TL1A protein can regulate the differentiation and activation of T cells. *TNFSF15* gene polymorphisms have been found to be associated with several autoimmune disorders. A possible association of *TNFSF15* with acute anterior uveitis (AAU) has not yet been reported and was therefore the purpose of our study.

Methods. Eight single nucleotide polymorphisms (SNPs) were examined using TaqMan SNP Genotyping Assay or PCR-restriction fragment length polymorphism in 983 AAU patients and 1128 healthy controls. Genotype distributions and allele frequencies were compared using χ^2 analysis between AAU patients and healthy controls. Stratified analysis was also performed according to ankylosing spondylitis (AS) status. The *TNFSF15* mRNA expression was quantified by real-time PCR.

Results. A significantly decreased frequency of the TT genotype in *TNFSF15*-rs3810936 was found in AAU patients ($P = 6.36 \times 10^{-6}$, corrected $P[Pc] = 1.52 \times 10^{-4}$, OR = 0.6, 95% CI = 0.5–0.8). Stratification according to AS status did not reveal a difference concerning the association with *TNFSF15*-rs3810936. None of the other *TNFSF15* SNPs tested were associated with AAU.

Conclusions. This study shows an association between *TNFSF15*-rs3810936 and AAU and suggests that the TL1A/DR3 pathway may be implicated in the pathogenesis of this disease.

Keywords: acute anterior uveitis, *TNFSF15*, ankylosing spondylitis

Uveitis is a serious intraocular inflammatory disease that can cause significant visual impairment. The prevalence of uveitis varies among different geographical locations across the world: 200 per 100,000 in the United States,[1] 40.4 per 100,000 in Japan,[2] 310 to 730 per 100,000 in Southern India,[3] and 111.3 per 100,000 in Taiwan.[4] Uveitis can present as an acute, recurrent, or chronic ocular inflammatory disease.[5] Four major types of uveitis are recognized, including anterior uveitis, intermediate uveitis, posterior uveitis, and panuveitis, a subdivision that is based on the anatomical localization within the eye.[6] The most common anatomic type is anterior uveitis, which accounts for nearly 78% of all cases in the Chinese population.[4] An aberrant autoimmune or autoinflammatory response mediated by T cells is thought to play an important role in the pathogenesis of various uveitis entities.[7]

Anterior uveitis is often associated with autoinflammatory conditions, such as ankylosing spondylitis (AS). Acute anterior uveitis (AAU) is a specific uveitis phenotype with sudden onset that occurs in 20% to 30% of AS patients and is also strongly associated with HLA-B27, suggesting a shared etiology with AS.[8–11] Recent findings show that various other genetic factors besides HLA-B27 are also associated with AAU and AS.[12] AAU and AS have been shown to share certain genetic associations, although some genes seem to be unique for either disease.[13] Variants in several genes, such as *FoxO1,*[14] *TRAF5,*[15] *IL-10,*[16] *IL-18R1,*[13] *CFB, CFH, C2,*[17,18] and *TNF,*[19,20] have recently been found to be associated with AAU. It is expected that with time more genes will be discovered that play a role in the predisposition to AAU.

Recently attention was drawn to the association of autoinflammatory diseases with genetic variants of the TNF superfamily member 15 (*TNFSF15*) gene region.[21–23] The *TNFSF15* gene encodes a protein called TNF-like cytokine 1A (TL1A).[21] Expression of TL1A by antigen presenting cells and other nonimmune cells is triggered by Toll-like receptors, FcγR crosslinking, and TNF or IL-1 stimulation. Increased local and systemic levels of the TL1A protein have been observed in several diseases, such as inflammatory bowel disease, psoriasis, and rheumatoid arthritis.[24–27] The TL1A can interact as a ligand with death receptor 3 (DR3), that is expressed on Tregs, Th1, Th17, and NKT cells, subsequently leading to activation of these cells, thereby spanning the innate and adaptive immune responses.[21] The term death receptor is misleading because most of the functions of this receptor are not related to cell death but to its effects on T-cell proliferation and differentiation. Of interest is the observation that DR3 mRNA and protein expression on $CD4^+$ T cells obtained from the lymph nodes of animals undergoing experimental autoimmune uveitis (EAU)

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Table 1. Primers and Restriction Enzymes Used for RFLP Analysis

SNP	Primers	Restriction Enzyme
rs4246905	5′-GGGAAACTGTAGACTTTGCTTAAA-3′ 3′-TTCATTCCTCCCCAAAGCAGT-5′	HpyF10VI (MwoI)
rs6478108	5′-TCAAAGTCCTAACTTATCCCAGTC-3′ 3′-CCATTTCTGGGCCAGGTCTC-5′	Alw26I (BsmAI)
rs4979462	5′-TTTTATGAGCAATGGCATCTGG-3′ 3′-TCCTGGCCAGTTTCAAAGCA-5′	BsuRI (HaeIII)
rs10817669	5′-GCTCCCTGTAGCCAACTTGT-3′ 3′-TGATGCCCTCGTCCTCCTAA-5′	Alw26I (BsmAI)
rs6478106	5′-CCAGTTTGGCAGACCCG-3′ 3′-CATTGCTTCCACTCCCCCTC-5′	AluI
rs10759734	5′-ACTTTAAATTATGAAGAATCACAT-3′ 3′-GTACTTACTGCTGAAAGACG-5′	HhaI
rs3810936	5′-GGAACCAGTTGCTACCTACTTCGTA-3′ 3′-CCGTGGGATGACCTCTGAGTG-5′	RsaI

was markedly enhanced, suggesting a role for the TL1A /DR3 pathway in the pathogenesis of this disease.[28] This was confirmed by studies showing that the EAU disease score was significantly decreased in DR3 knockout mice.[29]

The role of TL1A and DR3 in the pathogenesis of clinical autoinflammatory diseases has been investigated by analyzing polymorphisms in the *TNFSF15* gene region.[21,30–32] An association of *TNFSF15* with clinical uveitis has to our knowledge not yet been addressed and was therefore the purpose of the study described here, whereby we analyzed a large population of Chinese Han patients with acute anterior uveitis.

Materials and Methods

Study Population

A total of 983 AAU patients (498 $AAU^{+}AS^{+}$ and 485 $AAU^{+}AS^{-}$) were recruited for this project. The healthy control group comprised 1128 unrelated healthy subjects with no history of any ocular disease or autoimmune disease who were recruited from the same regions as the AAU group. All patients and controls were Han Chinese. There were no differences in age and sex distribution between the two groups. Both the patients and controls were recruited from the Uveitis Study Center of Sun Yat-sen University, Guangzhou, China, or the First Affiliated Hospital of Chongqing Medical University, Chongqing, China, from October 2006 to December 2014. The definition of AAU was based on the classification described by Jabs et al.,[6] and AAU was defined as AU with a duration of less than 3 months. The modified New York Criteria were used to diagnose AS.[33] All the subjects gave their informed consent before blood collection in accordance with the study protocol. The procedures of this study followed the Declaration of Helsinki. Our research was approved by the Ethics Research Committee of our hospital.

Genotyping and Single Nucleotide Polymorphism (SNP) Selection

Genomic DNA samples were extracted from peripheral blood leukocytes by using a commercially available kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The DNA content of the samples was quantified with a Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20°C until use.

Based on the results of previous studies,[22,30,34–40] we selected eight SNPs (rs3810936, rs4246905, rs6478108, rs4979462, rs10817669, rs10759734, rs10733612, rs6478106) of *TNFSF15* as candidate SNPs for our study. Samples were genotyped by PCR–restriction fragment length polymorphism (RFLP) for rs4246905, rs6478108, rs4979462, rs10817669, rs6478106, rs10759734, and rs3810936 (primers presented in Table 1), or by the Taqman SNP genotyping method for rs10733612 (C_31781327_10; Applied Biosystems, Foster City, CA, USA).

The PCR reaction was performed for 32 to 35 cycles with the following conditions: 95°C denaturation for 5 minutes, 95°C for 30 seconds, 56 to 64°C for 30 seconds, 72°C for 30 seconds, and followed by 72°C for 5 minutes. After digesting the PCR products with restriction enzymes (Thermo Fisher Scientific, Vilnius, Lithuania, Table 1) for at least 12 hours, the digested DNA was visualized on 3% to 5% agarose gels by staining with GoldView (SBS Genetech, Beijing, China). Genotyping errors were not observed after direct sequencing from randomly chosen samples (10% of all samples) by Biomed Co., Ltd. (Beijing, China). We obtained an overall genotyping call success rate of 98% or more and an accuracy of more than 99% in all the subjects.

Real-Time PCR

Peripheral blood mononuclear cells (PBMCs) were obtained from freshly heparinized venous blood samples of normal controls by Ficoll-Hypaque density gradient centrifugation. Total RNA was extracted from nonstimulated PBMCs and PBMCs stimulated by anti-CD3 antibody (5 μg/mL; eBioscience, San Diego, CA, USA) and anti-CD28 antibody (1 μg/mL, eBioscience) for 72 hours using a commercial reagent (TRIzol; Life Technologies, Grand Island, NY, USA), followed by reverse transcription using a commercial kit (Applied Biosystems). Real-time quantitative PCR was performed using the 7500 real-time instrument (ABI) with the following primers: TNFSF15, forward primer, 5′-GCAGGACTCACCACATACC-3′; reverse primer, 5′-CCTTGGCTTATCTCCGTCTG-3′, according to the SYBR-Green method. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Methods

The χ^2 test was used to evaluate Hardy-Weinberg equilibrium (HWE) for each SNP. Allele frequencies and genotype distributions were compared between the cases and the controls using the χ^2 analysis with SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA). Linkage disequilibrium (LD) was measured with Haploview (V4.0; Daly lab at the Broad Institute, Cambridge, MA, USA). The Bonferroni test was used to correct for multiple comparisons, and a *Pc* less than 0.05 was considered statistically significant.

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Table 2. Clinical Features of the Investigated AAU Patients and Controls

Clinical Features	*n*	%
AAU patients	983	100
Mean age ± SD, y	39.7 ± 12.3	
Male	607	61.7
Female	376	38.3
AAU with AS	498	50.7
AAU without AS	485	49.3
HLA-B27+ AAU	587 (792 tested)	74.1
HLA-B27+ AAU AS+	388 (428 tested)	90.7
HLA-B27+ AAU AS−	199 (364 tested)	54.7
HLA-B27− AAU	205 (792 tested)	25.9
HLA-B27− AAU AS+	40 (428 tested)	9.3
HLA-B27− AAU AS-	165 (364 tested)	45.3
FFA(+)	111 (248 tested)	44.8
Controls	1128	100
Mean age ± SD, y	39.6 ± 10.5	
Male	650	57.6
Female	478	42.4

Results

Clinical Features

In total we recruited 983 patients with AAU, including 607 (61.7%) males and 376 (38.3%) females. The mean age ± SD of the AAU patients was 39.7 ± 12.3 years. A total of 792 AAU patients were tested for HLA-B27, of whom 587 (74.1%) were positive and 205 (25.9%) were negative. A total of 498 (50.7%) of 983 patients were diagnosed with AS. The control subjects included 650 (57.6%) males and 478 (42.4%) females, and the average age was 39.6 ± 10.5 years. The detailed clinical characteristics are shown in Table 2.

Allele and Genotype Frequencies

A total of 983 AAU patients and 1128 healthy controls were genotyped for eight SNPs. All these SNPs were successfully genotyped, and none deviated significantly from HWE. There was a significant increase in the CT genotype frequency of *TNFSF15*-rs3810936 in AAU patients ($P = 3.96 \times 10^{-7}$, $Pc = 9.51 \times 10^{-6}$, odds ratio [OR] = 1.6, 95% confidence interval [CI] = 1.3–1.9) compared with healthy controls. There was a significant decrease in the TT genotype frequency of *TNFSF15*-rs3810936 in AAU patients ($P = 6.36 \times 10^{-6}$, $Pc = 1.52 \times 10^{-4}$, OR = 0.6, 95% CI = 0.5–0.8). There was no significant difference between AAU patients and healthy controls concerning the genotype and allele frequencies of the remaining SNPs (Table 3). Furthermore, there was no significant difference between HLA-B27$^+$ AAU patients and controls concerning the genotype and allele frequencies of the tested SNPs (Supplementary Table S1). Linkage disequilibrium block was estimated for eight SNPs of *TNFSF15* using our data. This showed that rs10759734 and rs10733612 were in strong LD ($r^2 = 0.9$) and that there was no linkage between the other SNPs (Fig. 1).

Table 3. Allele and Genotype Frequencies of Tested TNFSF15 SNPs in AAU Patients and Controls

SNPs	Allele and Genotype	AAU ($n = 983$)	Controls ($n = 1128$)	*P*	*Pc*	OR	95% CI
rs3810936	C	937 (0.478)	1003 (0.446)	0.04	NS	1.1	1.0–1.3
	CC	169 (0.172)	220 (0.196)	0.17	NS	0.9	0.7–1.1
	CT	599 (0.611)	563 (0.5)	3.96×10^{-7}	9.51×10^{-6}	1.6	1.3–1.9
	TT	213 (0.217)	342 (0.304)	6.36×10^{-6}	1.52×10^{-4}	0.6	0.5–0.8
rs10759734	A	1630 (0.838)	1871 (0.832)	0.55	NS	1.1	0.9–1.2
	AA	680 (0.7)	779 (0.692)	0.72	NS	1.0	0.9–1.2
	AG	270 (0.278)	313 (0.278)	0.98	NS	1.0	0.8–1.2
	GG	22 (0.023)	33 (0.029)	0.34	NS	0.8	0.4–1.3
rs4246905	C	1256 (0.644)	1426 (0.64)	0.78	NS	1.0	0.9–1.2
	CC	403 (0.413)	471 (0.423)	0.66	NS	1.0	0.8–1.1
	CT	450 (0.462)	484 (0.434)	0.21	NS	1.1	0.9–1.3
	TT	122 (0.125)	159 (0.143)	0.24	NS	0.9	0.7–1.1
rs6478108	C	925 (0.472)	1101 (0.492)	0.21	NS	0.9	0.8–1.0
	CC	186 (0.19)	263 (0.235)	0.01	NS	0.8	0.6–0.9
	CT	553 (0.565)	575 (0.514)	0.02	NS	1.2	1.0–1.5
	TT	240 (0.245)	281 (0.251)	0.75	NS	1.0	0.8–1.2
rs4979462	C	1451 (0.743)	1626 (0.729)	0.33	NS	1.1	0.9–1.2
	CC	529 (0.541)	599 (0.537)	0.85	NS	1.0	0.9–1.2
	CT	393 (0.402)	428 (0.384)	0.39	NS	1.1	0.9–1.3
	TT	55 (0.056)	88 (0.079)	0.04	NS	0.7	0.5–1.0
rs10817669	A	1619 (0.833)	1844 (0.823)	0.41	NS	1.1	0.9–1.3
	AA	682 (0.702)	766 (0.684)	0.38	NS	1.1	0.9–1.3
	AG	255 (0.262)	312 (0.279)	0.41	NS	0.9	0.8–1.1
	GG	35 (0.036)	42 (0.037)	0.86	NS	1.0	0.6–1.5
rs6478106	C	1411 (0.728)	1608 (0.715)	0.36	NS	1.1	0.9–1.2
	CC	502 (0.518)	585 (0.52)	0.91	NS	1.0	0.8–1.2
	CT	407 (0.42)	438 (0.39)	0.16	NS	1.1	1.0–1.3
	TT	60 (0.062)	101 (0.09)	0.02	NS	0.7	0.5–0.9
rs10733612	C	1629 (0.834)	1853 (0.828)	0.62	NS	1.0	0.9–1.2
	CC	677 (0.693)	769 (0.687)	0.78	NS	1.0	0.9–1.2
	CT	275 (0.281)	315 (0.282)	1.0	NS	1	0.8–1.2
	TT	25 (0.026)	35 (0.031)	0.44	NS	0.8	0.5–1.3

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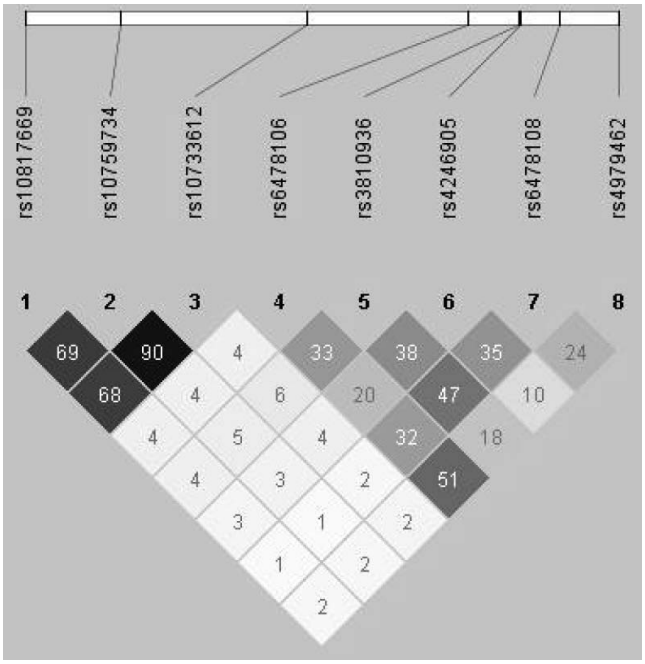

Figure 1. Linkage disequilibrium block was estimated for eight SNPs of TNFSF15 using our data. Numbers in the *squares* indicate correlation coefficient (r^2) values.

Association Between SNPs and AAU Stratified by AS Status

Among the 983 AAU patients, 498 patients (50.7%) were diagnosed with AS There was a significant increase in the CT genotype frequency of *TNFSF15*-rs3810936 in AAU^+AS^+ compared with healthy controls ($P = 2.66 \times 10^{-4}$, $Pc = 6.38 \times 10^{-3}$, OR = 1.5, 95% CI = 1.2–1.8). There was a significant decrease in the frequency of the *TNFSF15*-rs3810936 TT genotype frequency in AAU^+AS^+ patients ($P = 0.001$, $P = 0.024$, OR = 0.6, 95% CI = 0.5–0.8). There was a significant increase in the CT genotype frequency of *TNFSF15*-rs3810936 in AAU^+AS^- patients ($P = 6.03 \times 10^{-6}$, $Pc = 1.45 \times 10^{-4}$, OR = 1.7, 95% CI = 1.3–2.1) compared with healthy controls. There was a significant decrease in the frequency of the *TNFSF15*-rs3810936 TT genotype in AAU^+AS^- patients compared with healthy controls ($P = 1.92 \times 10^{-4}$, $Pc = 4.62 \times 10^{-3}$, OR = 0.6, 95% CI = 0.5–0.8) (Table 4). However, there was no significant difference between AAU^+AS^+ patients and AAU^+AS^- patients.

The Influence of rs3810936 on *TNFSF15* Expression

To determine whether rs3810936 was associated with *TNFSF15* expression, real-time PCR analysis was performed to evaluate whether this polymorphism affected the expression of the *TNFSF15* gene. Real-time PCR analysis did not reveal an effect of rs3810936 on the expression of *TNFSF15* in PBMCs of healthy controls ($P > 0.05$; Fig. 2). Furthermore, no difference of the *TNFSF15* mRNA expression in PBMCs stimulated with anti-CD3/CD28 antibodies was detected between the different genotypes ($P > 0.05$; Fig. 3).

Discussion

This study shows that gene variants of *TNFSF15* affect the predisposition to AAU in Chinese Han individuals. Individuals carrying the CT genotype of *TNFSF15* rs3810936 had a higher risk of developing AAU, whereas the TT genotype of this locus provided protection against AAU. Stratification of the patients on the basis of AS status showed that *TNFSF15*-rs3810936 was observed to be related to both groups of patients independent of whether they were also suffering from AS.

TNFSF15 is a member of the TNF family and via its gene product TL1A has an important impact on the major proinflammatory pathways (Th1, Th2, Th17, and Treg cells).[21] Our findings support earlier studies in the EAU model whereby mice with uveitis showed a higher expression of DR3 in lymph node cells and another study whereby mice deficient for TL1A had a markedly reduced disease severity.[28,41]

Various clinical studies support a role for the TL1A/DR3 pathway in several immune disorders. Both local and systemic levels of TLA1[21] were found to be markedly increased in diseases such as rheumatoid arthritis, psoriasis, primary biliary cirrhosis, inflammatory bowel disease (IBD), and AS.[27] Treatment of rheumatoid arthritis patients with TNF-alpha blocking antibodies was shown to be associated with a decrease in their serum TL1A levels.[42] Duplication of DR3 gene occurs more often in rheumatoid arthritis patients than in healthy controls.[43] In human IBD, the expression of DR3 in circulating T lymphocytes as well as in intestinal tissue was found to be increased.[44] Similar observations were made in psoriasis, where DR3 expression was also increased in affected skin.[25] The role of TL1A and DR3 in autoinflammatory disease was further supported by the finding that *TNFSF15* gene polymorphisms were associated with susceptibility to several diseases, such as IBD, AS, and irritable bowel syndrome.[37,45–49]

Genome-wide association studies reported associations between CD and variants in *TNFSF15*-rs6478106 in the Japanese population ($P = 3.87 \times 10^{-45}$).[22] A Korean study found that *TNFSF15* gene polymorphisms of rs3810936 and rs6478108 were associated with susceptibility to CD ($P = 4.4 \times 10^{-8}$, OR = 2.81, 95% CI = 1.94–4.07; $P = 2.7 \times 10^{-11}$, OR = 3.49, 95% CI = 2.42–5.04, respectively).[35] Similar results were recently observed in Indian patients with IBD.[47] This study is in agreement with previous studies cited above that also showed a protective effect of the rs3810936 TT genotype. In our study, TT occurred with a frequency of 21.7% in AAU as compared with controls who had a frequency of 30.4% ($P = 6.36 \times 10^{-6}$, $Pc = 1.52 \times 10^{-4}$, OR = 0.6, 95% CI = 0.5–0.8). These results suggest that *TNFSF15* gene polymorphisms may be shared risk factors for multiple diseases, including CD, IBD, and AAU.[31,32,34,35,46,49]

Table 4. Association Between *TNFSF15*-rs3810936 and AAU Stratified by AS Status

Allele and Genotype	AAU^+AS^+ ($n = 498$)	AAU^+AS^- ($n = 485$)	Controls ($n = 1128$)	*P*, AS^+	*Pc*, AS^+	OR	95% CI	*P*, AS^-	*Pc*, AS^-	OR	95% CI
C	478 (0.48)	459 (0.475)	1003 (0.446)	0.07	NS	1.1	1.0–1.3	0.13	NS	1.1	1.0–1.3
CC	90 (0.181)	79 (0.164)	220 (0.196)	0.48	NS	0.9	0.7–1.2	0.13	NS	0.8	0.6–1.1
CT	298 (0.598)	301 (0.623)	563 (0.5)	2.66×10^{-4}	6.38×10^{-3}	1.5	1.2–1.8	6.03×10^{-6}	1.45×10^{-4}	1.7	1.3–2.1
TT	110 (0.221)	103 (0.213)	342 (0.304)	0.001	2.40×10^{-2}	0.6	0.5–0.8	1.92×10^{-4}	4.62×10^{-3}	0.6	0.5–0.8

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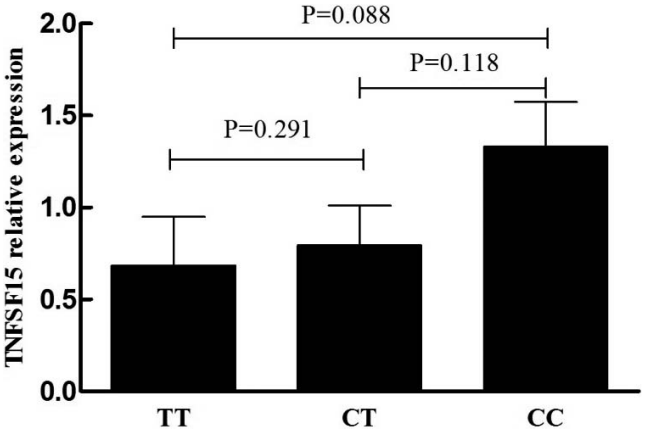

FIGURE 2. The influence of various rs3810936 genotypes (TT = 12, CT = 10, CC = 9) on the expression of *TNFSF15* in nonstimulated PBMCs. Expression of *TNFSF15* was not significantly different between genotypes. Data are expressed as mean ± SD.

A previous study reported that there was a direct association between genetic variants of the *TNFSF15* gene and the expression of TL1A protein in Jewish CD patients, which suggested that there was a significantly increased expression of membrane and soluble TL1A in response to immune complexes in TNFSF15 (TL1A) risk haplotype B–positive individuals. However, the membrane expression of TL1A was not increased in non-Jewish patients with the risk haplotype.[46] Therefore, the effects of TNFSF15 may depend on the ethnic background of the studied population.[46,49] Functional experiments in our study did not show an association between the different genotypes of rs3810936 and the expression of the *TNFSF15* gene and rs3810936 may be involved in AAU through other mechanisms rather than directly regulating *TNFSF15* gene transcription.

There are several limitations in our study that should be mentioned. First, our study was performed in a Han Chinese population and confirmation is necessary in other ethnic populations. Additionally, we recruited only uveitis patients in our study and further analysis of an AS patient group without uveitis is necessary to determine whether the association with rs3810936 is confined to AAU in a Chinese Han population. It is noteworthy to mention here that a comparison of HLA-B27–positive AS patients with HLA-B27 healthy controls did not reveal a significant association with *TNFSF15* gene polymorphisms in a Caucasian population.[50] It is not clear whether some of these AS patients also suffered from uveitis. A further limitation is the fact that our study was restricted to AAU patients and should therefore not be extrapolated to other uveitis entities. Furthermore, additional studies are needed to examine the exact role of the TL1A/DR3 pathway in uveitis, for instance by investigating local and systemic expression of TL1A and DR3 in these patients.

In summary, our study shows that *TNFSF15*-rs3810936 affects susceptibility to AAU in a Chinese Han population, implicating the TL1A/DR3 pathway in the pathogenesis of this disease.

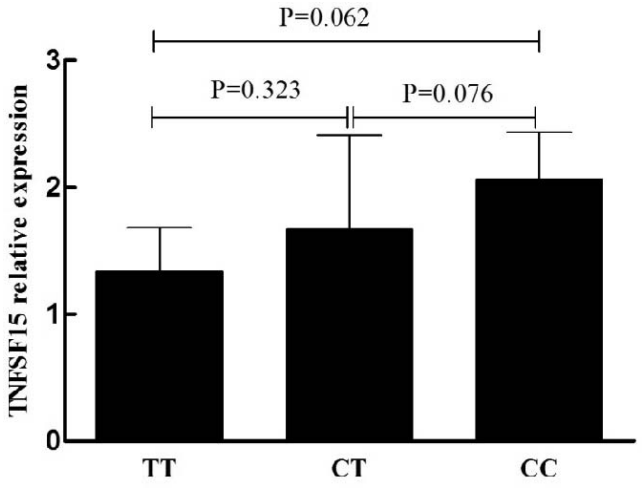

FIGURE 3. The influence of various rs3810936 genotypes (TT = 10, CT = 12, CC = 8) on expression of TNFSF15 in anti-CD3/CD28 antibodies in stimulated PBMCs. Expression of TNFSF15 was not significantly different between genotypes. Data are expressed as mean ± SD.

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