Genetics

FoxO1 Gene Confers Genetic Predisposition to Acute Anterior Uveitis With Ankylosing Spondylitis

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Purpose. Recent studies have shown that a decrease of regulatory T (Treg) cells may contribute to the activity of acute anterior uveitis (AAU) and ankylosing spondylitis (AS). A number of immunogenetic factors including *IL2RA*, *miR-27a*, *miR-182*, and *FoxO1* are associated with Treg cell function. In this study, we investigated the association between polymorphisms of these genes and AAU with or without AS in a Chinese Han population.

Methods. Using PCR-restricted fragment length polymorphism (RFLP) assay, a two-stage association study was performed in 680 AAU patients with or without AS and 1280 controls. Gene expression was quantified by real-time PCR.

Results. In the first stage study, an association analysis of 10 single nucleotide polymorphisms (SNPs) was performed in 230 AAU patients with AS, 240 AAU patients without AS, and 650 controls. The results showed significantly increased frequencies of the *FoxO1*/rs2297626 AA genotype and A allele in AAU patients with AS (AA genotype: $P = 6.23 \times 10^{-5}$, odds ratio [OR] = 1.86; A allele: $P = 2.17 \times 10^{-4}$, OR = 1.53). No significant association of the other 9 SNPs with AAU with or without AS was observed. In the second stage study, an association analysis of *FoxO1*/rs2297626 was performed in 210 AAU patients with AS and 630 controls. The second stage and combined studies confirmed the association of *FoxO1*/rs2297626 with AAU with AS (AA genotype: $P = 3.45 \times 10^{-8}$, OR = 1.85; A allele: $P = 1.55 \times 10^{-7}$, OR = 1.55).

Conclusion. This study suggests that *FoxO1*, but not *miR-27a*, *miR-182*, and *IL2RA*, contributes to the genetic susceptibility of AAU with AS, but none of the tested polymorphisms confer risk to AAU without AS.

Keywords: acute anterior uveitis, ankylosing spondylitis, *FoxO1*

Acute anterior uveitis (AAU) is one of the most common uveitis entities in the world.[1] The development of AAU may result in vision loss secondary to complicated cataract and glaucoma.[2] Previous reports have shown that 52% to 88% of patients with AAU are HLA-B27–positive according to different racial groups.[3–6] Besides, ankylosing spondylitis (AS) also is strongly associated with HLA-B27–positive AAU.[7,8] Recent studies have revealed that the concentration of regulatory T (Treg) cells inversely correlates with disease activity in HLA-B27–positive AAU and AS.[9,10] Moreover, recent surveys have shown that *TLR2*, *TLR4*, *TNF*, and *TRAF5* confer risk of AAU,[11–13] and that *IL-1A*, *ERAP1*, *IL-23R*, *ANTXR2*, *IL1R2*, *EDIL3*, *HAPLN1*, and *ANO6* polymorphisms are associated with AS.[14–18] These reports suggest that genetic factors besides the well-known HLA-B27 association may have an important role in both diseases.

The Treg cells are critical to the maintenance of immune cell homeostasis by negatively controlling a variety of physiological and pathological immune responses. Recent studies showed that the transcription factor forkhead-box O1 (*FoxO1*) has a key role in Treg cell function and may be involved in Treg-cell–associated immunological disorders.[19,20] The MiR-182, miR-27a, and miR-96 can reversely regulate FoxO1 expression by binding to its 3′ untranslated region.[21,22] Meanwhile, IL-2 receptor-α (IL2RA) has been identified to be responsible to induce miR-182.[22–24] Recently, polymorphisms of the genes regulating Treg function, such as *IL2RA*, *miR-182*, and *miR-27a*, have been considered as genetic predisposing factors involved in various immune diseases.[25–30] To our knowledge, no reports have appeared concerning the role of *FoxO1* gene polymorphisms and autoimmune-related diseases.

In view of the fact that these genes mentioned above have an important role in Treg cell development and function, we investigated whether polymorphisms of these genes were possibly associated with AAU with or without AS. Our results showed that *FoxO1*, but not *miR-27a*, *miR-182*, and *IL2RA*, contribute to the genetic susceptibility of AAU with AS, but none of the tested polymorphisms confer risk to AAU without AS.

Materials and Methods

Subjects

The study group consisted of 680 unselected, consecutive patients (440 AAU patients with AS and 240 AAU patients

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Table 1. Clinical Features of the Investigated AAU Patients

Clinical Features	*N*, Total = 680	Percentage
Mean age ± SD	39.3 ± 11.7	
Male	449	66.0
Female	231	34.0
Uveitis	680	100
AAU patients with AS	440	64.7
AAU patients without AS	240	35.3
HLA-B27^{+} AAU^{+}	451 (556 tested)	81.1
HLA-B27^{+} AAU^{+} AS^{+}	318 (355 tested)	89.6
HLA-B27^{+} AAU^{+} AS^{-}	133 (201 tested)	66.2
HLA-B27^{-} AAU^{+}	105 (556 tested)	18.9
HLA-B27^{-} AAU^{+} AS^{+}	37 (355 tested)	10.4
HLA-B27^{-} AAU^{+} AS^{-}	68 (201 tested)	33.8

without AS) who were recruited from the department of ophthalmology in the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) and the Zhongshan Ophthalmic Center of the Sun Yat-sen University (Guangzhou, China) between October 2006 and May 2014. A total of 1280 unselected, consecutive control subjects was matched geographically and ethnically with the patients. The AAU was diagnosed, as described by Jabs et al.,[31] as the presence of inflammatory cells in the anterior chamber and dust-like keratic precipitates (KPs) with flare, iridocyclitis, or iritis with a duration of less than 3 months. The AAU patients with other HLA-B27–associated diseases, such as inflammatory bowel disease, Reiter's syndrome, and psoriatic arthritis, were excluded from this study. The diagnosis of AS was based strictly on the modified New York Criteria for radiological sacroiliitis.[32] The study received the approval of the Local Ethics Research Committee (Permit Number 2009-201008), and all the tested subjects provided informed consent before blood collection. All procedures of this study followed the tenets of the Declaration of Helsinki.

DNA Extraction and Genotyping

Genomic DNA extraction of AAU patients and healthy controls was conducted using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The target DNA sequence was amplified by the PCR using proper primers as described previously.[33] The PCR products were digested with 3 units of restriction enzymes, such as PdiI, DraIII, AluI, Eco47I, BshNI, HindIII, TscAI, NdeI, BsmAI, BstNI (Fermentas, Shenzhen, China), and RsaI (Promega, Madison, WI, USA), in a 10 μL reaction volume for 14 hours. Digestion products were visualized on 3% or 4% agarose gels and stained with GoldView (SBS Genetech, Beijing, China).

Cell Isolation and Culture

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly drawn blood samples by Ficoll-Hypaque (TBDScience, Tianjin, China) density gradient centrifugation. Magnetic beads (Miltenyi Biotec, Palo Alto, CA, USA) were used to isolate CD4^{+} T cells according to the manufacturer's protocol. Purified CD4^{+} T cells were treated with anti-CD3/CD28 antibodies (5:1; Miltenyi Biotec) at 37°C for 72 hours.[33]

Real-Time PCR

Total RNA was extracted from CD4^{+} T cells and anti-CD3/CD28 antibodies-stimulated CD4^{+} T cells obtained from normal controls using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), followed by reverse transcription using a transcriptase kit (Applied Biosystems, ABI, Foster City, CA, USA). Real-time PCR was performed on the 7500 System (Applied Biosystems, ABI) based on the SYBR-Green method. The expression of *FoxO1* and β-actin (the internal reference) was examined using the primers as described previously.[33] All tests were conducted in triplicate, and relative expression levels were calculated and quantified by the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The χ^2 test was applied to analyze the Hardy-Weinberg equilibrium (HWE). Frequencies of genotype and allele were compared between patients and controls by the χ^2 test using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Odds ratios (OR) and 95% confidence intervals (CI) also were calculated using SPSS version 17.0 (SPSS, Inc.) to evaluate disease risk. *P* values were corrected for multiple comparisons with the Bonferroni correction method by multiplying with the number (40) of analyses performed, thus a *P* value less than 1.25×10^{-3} (0.05/40) was considered to be statistically significant. The nonparametric Mann-Whitney *U* test or independent samples *t*-test was used for comparing *FoxO1* expression levels among three genotype groups. A 2-tailed *P* value less than 0.05 was considered to be statistically significant.

Results

Clinical Features of AAU Patients

The detailed clinical features and demographic characteristics of the enrolled AAU patients are shown in Table 1. The distribution of genotype and allele frequencies of all the 10 SNPs investigated did not deviate from the Hardy-Weinberg equilibrium in the controls.

Genotype and Allele Frequencies of Tested SNPs in Controls and Patients in the First Stage Study

In total, 230 AAU patients with AS, 240 AAU patients without AS, and 650 controls were genotyped for 10 SNPs of *IL2RA*, *miR-27a*, *miR-182*, and *FoxO1* genes in the first stage study. Our results showed significantly increased frequencies of the *FoxO1*/rs2297626 AA genotype and A allele in AAU with AS patients (AA genotype: $P = 6.23 \times 10^{-5}$, OR = 1.86; A allele: $P = 2.17 \times 10^{-4}$, OR = 1.53; Table 2). However, lack of association between *FoxO1*/rs2297626 and AAU without AS was found, and none of the other 9 SNPs showed a significant association with AAU with or without AS (Supplementary Tables S1 and S2). Moreover, there was no significant association between the tested SNPs and HLA-B27 (Supplementary Table S3).

Genotype and Allele Frequencies of Tested SNPs in Controls and Patients in the Second Stage and Combined Studies

To validate the significant association between *FoxO1*/rs2297626 and AAU with AS found in the first stage, another 210 AAU patients with AS and 630 controls were enrolled for the second stage study. The results again revealed significantly increased frequencies of the *FoxO1*/rs2297626 AA genotype and A allele in AAU with AS (AA genotype: $P = 1.45 \times 10^{-4}$, OR = 1.84; A allele: $P = 1.94 \times 10^{-4}$, OR = 1.57; Table 2). The combined data confirmed the association between rs2297626 and AAU with AS (AA genotype: $P = 3.45 \times 10^{-8}$, OR = 1.85; A allele: $P = 1.55 \times 10^{-7}$, OR = 1.55; Table 2).

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TABLE 2. Main Effects of *FoxO1*/rs2297626 SNPs on AAU With AS Risk

Genotype	AAU With AS *N*	AAU With AS %	Controls *N*	Controls %	*P* Value	OR (95% CI)
Stage 1	$N = 230$		$N = 650$			
AA	114	49.5	225	34.6	6.23×10^{-5}*	1.86 (1.37–2.52)
AG	92	40.2	328	50.5	6.33×10^{-3}	0.65 (0.48–0.89)
GG	24	10.3	97	14.9	0.09	0.66 (0.41–1.07)
A allele	320	69.6	778	59.8	2.17×10^{-4}*	1.53 (1.22–1.93)
Stage 2	$N = 210$		$N = 630$			
AA	105	50.0	222	35.3	1.45×10^{-4}*	1.84 (1.34–2.52)
AG	85	40.3	313	49.6	0.02	0.69 (0.50–0.95)
GG	20	9.7	95	15.1	0.04	0.59 (0.36–0.99)
A allele	295	70.2	757	60.1	1.94×10^{-4}*	1.57 (1.24–1.99)
Combined	$N = 440$		$N = 1280$			
AA	219	49.8	447	34.9	3.45×10^{-8}*	1.85 (1.48–2.30)
AG	177	40.2	641	50.1	3.58×10^{-4}*	0.67 (0.54–0.84)
GG	44	10.0	192	15.0	8.55×10^{-3}	0.63 (0.45–0.89)
A allele	615	69.9	1535	60.0	1.55×10^{-7}*	1.55 (1.32–1.83)

* *P* value with Bonferroni correction less than 1.25×10^{-3} was considered to be significant.

The Influence of rs2297626 on *FoxO1* Expression

The aforementioned result showed a significant association between *FoxO1*/rs2297626 and AAU with AS. To investigate a possible function associated with this SNP, we performed real-time PCR analysis to evaluate its effect on the expression of *FoxO1* using $CD4^+$ T cells derived from 30 healthy individuals with known genotype. The results did not reveal an effect of the various rs2297626 genotypes on *FoxO1* expression (see Fig., $P > 0.05$). We subsequently examined whether the expression of *FoxO1* was affected by the various rs2297626 genotypes in anti-CD3/CD28 antibodies-stimulated $CD4^+$ T cells. Although the results showed a lower *FoxO1* expression in the rs2297626 AA genotype compared to the GG genotype in anti-CD3/CD28 antibodies-stimulated $CD4^+$ T cells, no statistically significant differences were found between the two groups (see Fig., $P > 0.05$).

Discussion

In the present study, we showed that a gene polymorphism in *FoxO1* confers risk to AAU in combination with AS. Other polymorphisms in genes also affecting the function of Tregs, such as *miR-27a*, *miR-182*, and *IL2RA*, did not contribute to the genetic susceptibility of AAU with AS. None of the investigated gene polymorphisms was associated with AAU in the absence of AS. Up to now, to our knowledge no common SNP (minor allele frequency > 0.05) has been found in miR-96, which also is a pivotal regulator of *FoxO1* function and this factor, therefore, was not included in our study.

The IL2RA, also known as CD25, has a key role in the regulation of the immune system, and is responsible for the induction of miR-182 by activating STAT5. Previous studies suggested that polymorphisms of the *IL2RA* gene are associated with a variety of autoimmune-related diseases, such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes.[28,34,35] A recent study did not detect association between several SNPs of *IL2RA* (rs2104286, rs11594656, and rs12722495) and endogenous nonanterior uveitis.[36] In this study, we also did not find an association between *IL2RA* and AAU with or without AS. This finding is in agreement with our recent study that SNPs for *IL2RA* (rs706778, rs3118470, rs2104286, and rs7093069) were not associated with either Behçet's disease (BD) or Vogt-Koyanagi-Harada (VKH) syndrome.[33]

The MiR-182 has a pivotal role in modulating adaptive immune responses by binding to a specific site of 3′ untranslated region of *FoxO1* gene.[23,37,38] Earlier polymorphism analysis identified a significant association between *miR-182*/rs76481776 and late insomnia in major depression patients.[29] Our recent study also showed that the CC genotype of *miR-182*/rs76481776 had a significantly decreased frequency in BD and VKH patients, and a significantly increased expression of *miR-182* in rs76481776 TT/CT cases compared to CC cases in anti-CD3/CD28 antibodies-stimulated $CD4^+$ T cells.[33] However, no significant association between *miR-182*/rs76481776 and AAU with or without AS was found in this study.

Also, MiR-27a can bind to 3′ untranslated region of *FoxO1* and negatively regulate its expression, resulting in abnormal differentiation and function of Treg cell.[21,22] Recent surveys have shown that *miR-27a*/ rs895819 is associated with various cancers, such as gastric cancer, renal cell cancer, and breast cancer.[26,39,40] In this study, we did not find an association between *miR-27a*/rs895819 and AAU with or without AS, which is in agreement with our recent study that *miR-27a*/rs895819 was not associated with BD or VKH.[33]

The *FoxO1* controls the development and function of Treg cells by binding to the promoter regions of *Foxp3* and *CTLA-4* genes.[19,20] Previous surveys suggested that SNPs of *FoxO1* were not associated with type 2 diabetes.[41] Our recent study also didn't find a direct association between SNPs of *FoxO1* with uveitis in either BD or VKH.[33] To the best of our knowledge, the association between *FoxO1*/rs2297626 polymorphisms and autoimmune-related diseases has not yet been reported, and as yet there are no published genome-wide association studies (GWAS) for AAU. Although there are several published GWAS for AS,[17,18] including Chinese datasets, no evidence of an association between *FoxO1*/rs2297626 and AS was reported. In this study, our result showed that the AA genotype of *FoxO1*/rs2297626 was significantly increased in frequency in AAU patients with AS, whereas there was no significant association between *FoxO1*/rs2297626 and AAU without AS. Whether *FoxO1/rs2297626* is associated with AS alone or the combination of AAU and AS is not clear and is expected to be elucidated in the future. Although a trend for a

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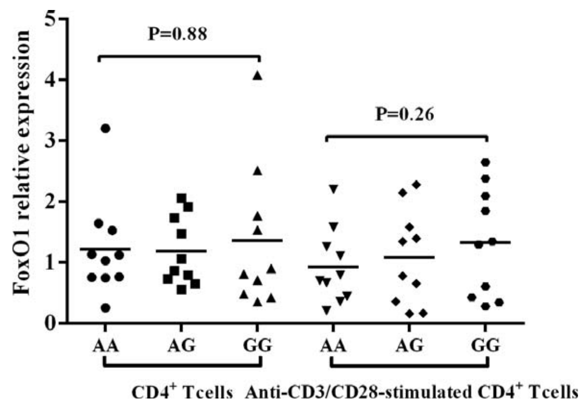

Figure. The influence of various rs2297626 genotypes on expression of *FoxO1*. Purified $CD4^+$ T cells and anti-CD3/CD28 antibodies-stimulated $CD4^+$ T cells from genotyped healthy controls were used (10 AA genotypes, 10 AG genotypes, and 10 GG genotypes of rs2297626).

lower expression of *FoxO1* was observed in rs2297626 AA cases compared to GG cases in anti-CD3/CD28 antibodies-stimulated $CD4^+$ T cells, data did not reach statistical significance. In view of the small sample size, further studies are needed to investigate whether polymorphisms of *rs2297626* can influence the expression of the *FoxO1* gene. The fact that *FoxO1* is critical for Treg cell function may expand our knowledge concerning the role of these cells in the pathogenesis of AAU with AS.

Our study has a number of limitations. Firstly, as our study only recruited patients visiting an ophthalmology department, only uveitis patients were included; thus, whether the same association exists between *FoxO1* gene and AS patients without uveitis deserves further study. Furthermore only, a limited number of SNPs of *IL2RA* and *FoxO1* genes were tested in our study and it is possible that other as yet unknown SNPs also might be involved. This survey was performed in Han Chinese, and future studies are needed in other ethnic populations to confirm the results.

Conclusions

Our results suggested that *FoxO1*, but not *miR-27a*, *miR-182*, and *IL2RA*, contributes to the genetic susceptibility to AAU with AS. Further research concerning the role of *FoxO1* and the biochemical pathways that control T cell homeostasis are needed to elucidate their role in the development of AAU with AS.

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References

1. Chang JH, Wakefield D. Uveitis: a global perspective. *Ocul Immunol Inflamm*. 2002;10:263–279.
2. Nussenblatt RB, Gery I. Experimental autoimmune uveitis and its relationship to clinical ocular inflammatory disease. *J Autoimmun*. 1996;9:575–585.
3. Brewerton D, Caftrey M, Nicholls A. Acute anterior uveitis and HLA B27. *Lancet*. 1973;2:994–996.
4. Saari R, Lahti R, Saari KM, et al. Frequency of rheumatic diseases in patients with acute anterior uveitis. *Scand J Rheumatol*. 1982;11:121–123.
5. Torres S, Borges S, Artiles A. HLA-B27 and clinical features of acute anterior uveitis in Cuba. *Ocul Immunol Inflamm*. 2013; 21:119–123.
6. Yang P, Zhang Z, Zhou H, et al. Clinical patterns and characteristics of uveitis in a tertiary center for uveitis in China. *Curr Eye Res*. 2005;30:943–948.
7. Beckingsale A, Davis J, Gibson J, Rosenthal A. Acute anterior uveitis, ankylosing spondylitis, back pain, and HLA B27. *Br J Ophthalmol*. 1984;68:741–745.
8. Banares A, Garcia H, Fernandez GB. Eye involvement in the spondyloarthropathies. *Rheum Dis Clin North Am*. 1998;24: 771–784.
9. Zou W, Wu Z, Xiang X, Sun S, Zhang J. The expression and significance of T helper cell subsets and regulatory T cells CD(4)(+) CD(2)(5)(+) in peripheral blood of patients with human leukocyte antigen B27-positive acute anterior uveitis. *Graefes Arch Clin Exp Ophthalmol*. 2014;252:665–672.
10. Zhao SS, Hu JW, Wang J, Lou XJ, Zhou LL. Inverse correlation between CD4+ CD25high CD127low/- regulatory T-cells and serum immunoglobulin A in patients with new-onset ankylosing spondylitis. *J Int Med Res*. 2011;39:1968–1974.
11. Chang JH, Hampartzoumian T, Everett B, Lloyd A, McCluskey PJ, Wakefield D. Changes in Toll-like receptor (TLR)-2 and TLR4 expression and function but not polymorphisms are associated with acute anterior uveitis. *Invest Ophthalmol Vis Sci*. 2007;48:1711–1717.
12. Kuo NW, Lympany PA, Menezo V, et al. TNF-857T, a genetic risk marker for acute anterior uveitis. *Invest Ophthalmol Vis Sci*. 2005;46:1565–1571.
13. 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.
14. Maksymowych WP, Rahman P, Reeve JP, Gladman DD, Peddle L, Inman RD. Association of the IL1 gene cluster with susceptibility to ankylosing spondylitis: an analysis of three Canadian populations. *Arthritis Rheum*. 2006;54:974–985.
15. Davidson SI, Wu X, Liu Y, et al. Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. *Arthritis Rheum*. 2009;60:3263–3268.
16. Rahman P, Inman RD, Gladman DD, Reeve JP, Peddle L, Maksymowych WP. Association of interleukin-23 receptor variants with ankylosing spondylitis. *Arthritis Rheum*. 2008; 58:1020–1025.
17. Lin Z, Bei JX, Shen M, et al. A genome-wide association study in Han Chinese identifies new susceptibility loci for ankylosing spondylitis. *Nat Genet*. 2012;44:73–77.
18. Australo-Anglo-American Spondyloarthritis Consortium (TASC), Reveille JD, Sims AM, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet*. 2010;42:123–127.

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19. Kerdiles YM, Stone EL, Beisner DR, et al. Foxo transcription factors control regulatory T cell development and function. *Immunity.* 2010;33:890–904.
20. Ouyang W, Liao W, Luo CT, et al. Novel Foxo1-dependent transcriptional programs control T(reg) cell function. *Nature*. 2012;491:554–559.
21. Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. *J Biol Chem*. 2009;284:23204–23216.
22. O'Neill LA. Outfoxing Foxo1 with miR-182. *Nat Immunol*. 2010;11:983–984.
23. Stittrich AB, Haftmann C, Sgouroudis E, et al. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. *Nat Immunol*. 2010;11: 1057–1062.
24. Kelada S, Sethupathy P, Okoye IS, et al. miR-182 and miR-10a are key regulators of Treg specialisation and stability during Schistosome and Leishmania-associated inflammation. *PLoS Pathogens*. 2013;9:e1003451.
25. Mussig K, Staiger H, Machicao F, et al. Association of common genetic variation in the FOXO1 gene with beta-cell dysfunction, impaired glucose tolerance, and type 2 diabetes. *J Clin Endocrinol Metab*. 2009;94:1353–1360.
26. Sun Q, Gu H, Zeng Y, et al. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. *Cancer Sci*. 2010;101: 2241–2247.
27. Klinker MW, Schiller JJ, Magnuson VL, et al. Single-nucleotide polymorphisms in the IL2RA gene are associated with age at diagnosis in late-onset Finnish type 1 diabetes subjects. *Immunogenetics*. 2010;62:101–107.
28. Kawasaki E, Awata T, Ikegami H, et al. Genetic association between the interleukin-2 receptor-alpha gene and mode of onset of type 1 diabetes in the Japanese population. *J Clin Endocrinol Metab*. 2009;94:947–952.
29. Saus E, Soria V, Escaramis G, et al. Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. *Hum Mol Genet*. 2010;19:4017–4025.
30. Hinks A, Cobb J, Sudman M, et al. Investigation of rheumatoid arthritis susceptibility loci in juvenile idiopathic arthritis confirms high degree of overlap. *Ann Rheum Dis*. 2012;71: 1117–1121.
31. Jabs DA, Nussenblatt RB, Rosenbaum JT. The Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol*. 2005; 140:509–516.
32. Van der Linden S, Valkenburg H, Cast A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. *Arthritis Rheum*. 1984; 27:361–368.
33. Yu H, Liu Y, Bai L, Kijlstra A, Yang P. Predisposition to Behcet's disease and VKH syndrome by genetic variants of miR-182. *J Mol Med (Berl)*. 2014;92:961–967.
34. Knevel R, de Rooy DP, Zhernakova A, et al. Association of variants in IL2RA with progression of joint destruction in rheumatoid arthritis. *Arthritis Rheum*. 2013;65:1684–1693.
35. Schmied MC, Zehetmayer S, Reindl M, et al. Replication study of multiple sclerosis (MS) susceptibility alleles and correlation of DNA-variants with disease features in a cohort of Austrian MS patients. *Neurogenetics*. 2012;13:181–187.
36. Cenit MC, Marquez A, Cordero-Coma M, et al. Evaluation of the IL2/IL21, IL2RA and IL2RB genetic variants influence on the endogenous non-anterior uveitis genetic predisposition. *BMC Med Genet*. 2013;14:52.
37. Ceribelli A, Satoh M, Chan EK. MicroRNAs and autoimmunity. *Curr Opin Immunol*. 2012;24:686–691.
38. Zhu S, Pan W, Qian Y. MicroRNA in immunity and autoimmunity. *J Mol Med (Berl)*. 2013;91:1039–1050.
39. Shi D, Li P, Ma L, et al. A genetic variant in pre-miR-27a is associated with a reduced renal cell cancer risk in a Chinese population. *PLoS One*. 2012;7:e46566.
40. Zhang N, Huo Q, Wang X, et al. A genetic variant in pre-miR-27a is associated with a reduced breast cancer risk in younger Chinese population. *Gene*. 2013;529:125–130.
41. Li T, Wu X, Zhu X, et al. Association analyses between the genetic polymorphisms of HNF4A and FOXO1 genes and Chinese Han patients with type 2 diabetes. *Mol Cell Biochem*. 2011;353:259–265.

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