Association of GTF2I and GTF2IRD1 polymorphisms with systemic lupus erythematosus in a Chinese Han population

Y. Li[1,2], P. Li[1], S. Chen[1], Z. Wu[1], J. Li[1], S. Zhang[1], C. Cao[1], L. Wang[1], B. Liu[2], F. Zhang[1], Y.Z. Li[1]

[1]Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing, China;
[2]Department of Rheumatology, The Affiliated Hospital of Qingdao University Medical College, Shangdong, China.

ABSTRACT

Objective

Systemic lupus erythematosus (SLE) is the most common systemic autoimmune disease which likely involves complex interactions between genes and the environment. Two large-scale genome-wide association studies (GWAS) have implicated many loci as genetic risk factors associated with primary Sjögren's syndrome (pSS). Among them there are a number of pSS associated gene polymorphisms including the MHC-II, STAT4, IRF5, BLK, and TNIP1 genes that are shared with SLE. However, the association of other genes such as GTF2I, GTF2IRD1, and IL12A with SLE remain unknown. This study aimed to determine whether single nucleotide polymorphisms (SNPs) in GTF2I, GTF2IRD1 or IL12A genetically predispose a Chinese Han population to SLE.

Methods

Four SNPs in the GTF2I region (rs117026326), the GTF2IRD1 region (rs4717901), and the IL12A region (rs485497, rs583911) were genotyped in a cohort of 948 SLE patients and 938 healthy controls, using the polymerase chain reaction-ligation detection reaction (PCR-LDR) method.

Results

The frequency of risk allele of rs117026326 was notably higher in SLE patients than in controls (37.2% vs. 14.9%, OR: 3.39, 95%CI: 2.89-3.97, $p_c = 3.31 \times 10^{-54}$). Similarly, rs4717901 was also associated with SLE (35.3% vs. 20.2%, OR: 2.16, 95%CI: 1.86-2.50, $p_c = 1.50 \times 10^{-24}$). The frequencies of alleles and genotypes of IL12A SNPs were not significantly different between the SLE patients and controls.

Conclusion

This study demonstrates a significant association between SLE and the GTF2I rs117026326 T allele, GTF2IRD1 rs4717901 C allele. The association of GTF2I and GTF2IRD1 as common genetic susceptibility factor in SLE will require further validation in other ethnic lines.

Key words

systemic lupus erythematosus, single-nucleotide polymorphism, genetic susceptibility, GTF2I, GTF2IRD1

*Yuan Li, MM**
*Ping Li, MM**
*Si Chen, MM**
Ziyan Wu, MM
Jing Li, MM
Shulan Zhang, MM
Chunwei Cao, PhD
Li Wang, MD
Bin Liu, MD
Fengchun Zhang, MD
Yongzhe Li, MD

**These authors contributed equally to this work.*

Please address correspondence and reprint requests to:
Dr Yongzhe Li,
Department of Rheumatology and Clinical Immunology,
Peking Union Medical College and Chinese Academy of Medical Sciences,
41 Damucang Hutong,
Xicheng District,
100032 Beijing, China.
E-mail: yongzhelipumch@126.com

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Introduction

Systemic lupus erythematosus (SLE) is a chronic, systemic, inflammatory autoimmune disease that causes tissue or organ damage characterised by a diverse array of autoantibody production, complement activation and immune complex formation (1, 2). The aetiology and pathogenesis of SLE involves genetic, epigenetic, and environmental factors. Of note, genetic factors play an important role in the susceptibility to the disease. More than 100 possible genetic risk factors for SLE have been identified (3, 4).

Primary Sjögren's syndrome (pSS) shares many features with SLE including the postive of anti-SSA/SSB antibodies, a similar interferon-α gene signature, activation of interferon (IFN) pathways, recruitment of plasmacytoid dendritic cells, and other aspects of immune activation (5, 6). Several reports have demonstrated the coexistence of SLE and pSS. Patients with pSS can later develop SLE with secondary SS. Moreover, 17.8% of SLE patients presented with SS/SLE (7). SLE with secondary SS and pSS has a common genetic background (8-11), the same HLA alleles are associated with the anti-SSA/SSB antibodies presence pattern: HLA DRB1*15 associated with presence of anti-SSA antibody alone and DRB1*03 associated with secretion of both anti-SSA and anti-SSB antibody (12, 13).

Two recent large-scale genome-wide association studies (GWAS) involving Chinese populations led to the discovery and validation of multiple susceptibility loci for pSS (14, 15). Our previous GWAS showed that GTF2I, located at 7q21, was the most strongly associated gene in the Chinese Han pSS patients, and had higher OR scores than other pSS associated identified genes including MHC-II genes, STAT4, and TNFAIP3 (14). GTF2I encodes the general transcription factor IIi (TFII-I). TFII-I plays an important role in signal-induced transcriptional regulation of both B cells and T cells (16). Another susceptibility locus, GTF2IRD1, which its molecular function has not been fully studied, has been reported to be involved in mammalian craniofacial and cognitive development (17). Insufficiency of GTF2IRD1 protein contributes to abnormalities of facial development, motor function and specific behavioural disorders that accompany Williams-Beuren syndrome (18). GWAS found that seven variants in the IL12A (encoding interleukin-12A) region demonstrated strong association, with the peak association at rs485497, and only rs485497 was associated with IL12A transcript expression. IL12A gene encodes a subunit of a cytokine that has a critical role in the production of IFN-γ by T cells and natural killer cells and the differentiation of both Th1 and Th2 cells (19). So far, reports on IL12A region in SLE are absent. However, association of SNP variants within the 3′ end of the IL12A gene in primary biliary cirrhosis (PBC) and 5′ end of the IL12A gene in coeliac disease (20, 21). Other studies have revealed associations of non-Hodgkin's lymphoma (NHL) with an intron region SNP, rs485497, which plays a central role in bridging the cellular and humoral pathways of innate resistance and antigen-specific adaptive immune responses (22). Rs583911 has also been confirmed as associated with childhood acute lymphoblastic leukaemia (ALL) in a Chinese population (23).

Considering the genetic overlap in the autoimmune diseases and the associations of these genes with SLE in other populations, we hypothesised that some of the related polymorphisms of GTF2I, GTF2IRD1 and IL12A may also contribute to genetic susceptibility to SLE in a Chinese Han population. Then, we developed the first large case-control study to determine out the relationship between GTF2I, GTF2IRD1 and IL12A polymorphisms and SLE.

Materials and methods

Patients and controls

This study was designed as a case-control including 948 patients with SLE and 938 healthy unrelated ethnically-matched controls recruited and enrolled in the Rheumatology Department of Peking Union Medical College Hospital (PUMCH). All SLE patients met the 1997 American College of Rheumatology (ACR) classification criteria for lupus (24). As we all know,

anti-SSA/B antibodies were specific diagnostic markers of pSS. pSS patients commonly experience fatigue, pain and cognitive symptoms. Lupus nephritis was a manifestation of SLE resulting from glomerular immune complex deposition and inflammation. So, we stratified SLE patients based on 12 subphenotypes, including anti-SSA/B antibodies, anti-Sm antibodies, anti-RNP antibodies, anti-dsDNA antibodies, low C3 or C4 levels, nephritis, neurological disorder, arthritis, haematologic disorder, rash and SLE/pSS. In SLE patients, autoantibodies including anti-nuclear antibodies (ANA), anti-dsDNA, anti-SSA/B, anti-Sm, and anti-RNP antibodies were determined either by indirect immunofluorescence or double immunodiffusion analysis. In our study, there were 84 SLE patients presented with SS/SLE. Further, information of other clinical manifestations was also recorded for each patient (Table I). This study was approved by the ethics committee of the PUMCH, and all study participants provided informed consents.

Table I. Characteristics of the SLE patients and control subjects.

Characterisitic	Case (n)	Control (n)
Male/femal	85/863	84/854
Age, years (mean±SD)	36.34±12.97	36.75±10.91
anti-nuclear antibodies	928	—
anti-SSA antibodies*	454	—
anti-SSB antibodies*	105	—
anti-Sm antibodies*	184	—
anti-RNP antibodies*	270	—
anti-dsDNA antibodies	445	—
Low C3 or C4 levels	591	—
Nephritis	517	—
Neuropsychiatric disorder	138	—
Arthritis	381	—
Haematologic disorder	305	—
Rash	347	—
SLE/pSS	84	—

*Data were available for 923 patients.

Genotyping

Genomic DNA was extracted from 2 mL ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood samples by using DNA isolation kits from Bioteke (Beijing, China) following the manufacturer's instructions and was stored at -80 °C until used for genotype testing. SNP genotyping was performed using the PCR-LDR method (25, 26) with technical support from the Shanghai Biowing Applied Biotechnology company. The probe and primer sequences for the PCR-based ligase detection reaction in GTF2I, GTF2IRD1 and IL12A genes have been shown in supplementary material I and II. The target DNA sequences were amplified using a multiplex PCR method. The ligation reaction for each subject was carried out in a final volume of 20 ul reaction mixture containing 2 ul PCR buffer, 0.6 ul Mg^{2+}, 2 ul dNTP, 0.2 ul Qiagen HotStarTaq Polymerase (QIAGEN, Germany), 4 ul Q-solution, 2 ul Primer mix and 12.2 ul H_2O. This mixture was applied to a thermal cycler for amplification. Thermal cycling was performed in the Perkin-Elmer Gene Amp PCR Systems 9600. The amplification procedure consisted of initial denaturation at 95°C for 15 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 90 s and extension at 72°C for 60 s, followed by a final extension at 72°C for 7 min. The LDR was performed in a final volume of 10 ul reaction mixture containing 1 ul buffer, 1 ul probe mix, 0.05 ul Taq DNA ligase (New England Biolabs, Ipswich, MA, USA), 4 ul multi-PCR products and 4 ul H_2O. The LDR was performed using 35 cycles of denaturation at 95°C for 2 min, annealing at 94°C for 30 s and extension at 60°C for 2 min. The fluorescent products of LDR were differentiated by ABI sequencer 377. The quality of genotyping was controlled using 50 blinded blood duplicates.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested using Chi-square (χ^2) test. Any SNPs that deviated from HWE ($p<0.05$) were excluded from further analysis. Mann-Whitney U-test was used for evaluating the age distribution between cases and controls. Allele and genotype frequencies of cases and controls were calculated by χ^2-test using the PLINKv1.07 whole-genome data analysis toolset (http://pngu.mgh.harvard.edu/Bpurcell/plink/). The odds ratios (OR) of associations were calculated with 95% confidence intervals (95% CI). Based on different genetic models (additive, dominant, and recessive), association analyses were performed. The *p*-values were corrected (p_c) with the Bonferroni correction for multiple testing. $p_c<0.05$ was considered significant.

Results

Characteristics of participants

In this study, 948 SLE patients (male/female, 85/863) and 938 ethnically- and geographically-matched healthy controls (male/female, 84/854) were collected from a Chinese Han population. The demographic data and clinical features of SLE patients were illustrated in Table I. The ages of cases (36.34±12.97 years) and controls (36.75±10.91 years) matched well, according to the Mann-Whitney U-test ($p=0.06$). All the four polymorphisms were within Hardy-Weinberg equilibrium for the control group ($p>0.05$) and the call rate >95%. The accuracy was 100% as 50 samples were duplicately genotyped and the results were consistent.

Allele and genotype frequencies between cases and controls

The distribution of both allelic frequencies and genotypic frequencies of the four SNPs was shown in Table II. The frequency of rs117026326-T was higher in SLE patients than in controls (37.2% *vs*. 14.9%, OR=3.39, 95%CI: 2.89–3.97, $p_c=3.31\times10^{-54}$). Similarly, rs4717901 was also associated with SLE (35.3% *vs*. 20.2%, OR=2.16, 95%CI: 1.86-2.50, $p_c=1.50\times10^{-24}$). Statistical analysis using multiple logistic regressions in genetic additive, dominant, and recessive

Table II. Allele and genotype distribution of the GTF2I, GTF2IRD1 and IL12A gene markers in SLE patients and healthy controls.

Gene	SNP	Allelic test					Genotypic test				
		Allele	Case (n) / control (n)	p	p_c	OR(95% CI)	Genotype	Case (n) / control (n)	p	p_c	χ^2
GTF2I	rs117026326	T C	704/279 1190/1597	8.27×10^{-55}	3.31×10^{-54}	3.39(2.89-3.97)	T/T T/C C/C	138/15 428/249 381/674	3.88×10^{-50}	1.55×10^{-50}	227.5
GTF2IRD1	rs4717901	C A	669/379 1225/1497	3.74×10^{-25}	1.50×10^{-24}	2.16(1.86-2.5)	C/C C/A A/A	125/39 419/301 403/598	5.86×10^{-23}	2.34×10^{-22}	102.4
IL12A	rs485497	G A	466/503 1428/1373	0.12	0.48	0.89(0.77-1.03)	G/G G/A A/A	60/72 346/359 541/507	0.3	NS	2.391
IL12A	rs583911	A G	563/583 1331/1293	0.37	NS	0.94(0.82-1.08)	A/A G/A G/G	84/92 395/399 468/447	0.66	NS	0.82

SNP: single-nucleotide polymorphism; p_c: values after Bonferroni corrections; NS: not significant.

Table III. Analysis of the six SNPs based on three genetic models.

Gene	SNP	Additive model		Dominant model		Recessive model	
		p	OR(95%CI)	p	OR(95%CI)	p	OR(95%CI)
GTF2I	rs117026326	$1..39\times10^{-46}$	3.37 (2.85-3.98)	6.8×10^{-42}	3.79 (3.13-4.6)	1.67×10^{-17}	10.5 (6.11-18.03)
GTF2IRD1	rs4717901	4.5×10^{-23}	2.13 (1.83-2.47)	5.78×10^{-20}	2.37 (1.97-2.86)	3.75×10^{-11}	3.51 (2.42-5.08)
IL12A	rs485497	0.124	0.89 (0.77-1.03)	0.18	0.88 (0.74-1.06)	0.26	0.81 (0.57-1.16)
IL12A	rs583911	0.37	0.94 (0.82-0.90)	0.44	0.93 (0.78-1.11)	0.48	0.90 (0.66-1.22)

SNP: single-nucleotide polymorphism. *Bonferroni corrections data not shown.

models showed similar patterns (Table III). Rs117026326 and rs4717901 were associated with SLE in the additive model, dominant model and recessive model. Rs485497 and rs583911were not risk factors for SLE.

Correlation between SLE SNPs and the subphenotypes of SLE

We also examined the associations between the SNPs and various clinical manifestations of SLE. SNPs rs117026326 of GTF2I and rs4717901of GTF2IRD1 demonstrate a correlation with anti-SSA/B antibodies, anti-Sm antibodies, anti-RNP antibodies, anti-dsDNA antibodies, low C3 or C4 levels, nephritis, neurological disorder, arthritis, haematologic disorder, rash and SLE/pSS. Rs485497 of IL12A was associated with nephritis (OR=0.82, 95% CI: 0.69-0.98, p=0.03) and low C3 or C4 levels (OR=0.82, 95% CI: 0.69–0.97, p=0.02), but the association disappeared after Bonferroni correction (p_c=0.12 and p_c=0.08). No association was found between rs583911 and any clinical features (p>0.05). Risk alleles may correlate only with limited clinical features of the disease rather than general disease susceptibility.

Discussion

To our knowledge, this is the first report which indicates an influence of the GTF2I, GTF2IRD1 polymorphism in SLE in a well-defined cohort of chinese. According to two recently conducted GWAS, GTF2I, GTF2IRD1 and IL12A genes display the strong association with pSS. Our study confirmed that patients carrying GTF2I rs117026326-T and GTF2IRD1 rs4717901-C allele were at increased risk of developing SLE in Chinese Han. Moreover, rs117026326 and rs4717901 were also associated with SLE in different genetic models (additive model, dominant model and recessive model). SNPs rs117026326 and rs4717901 demonstrate a correlation with SLE subgroups stratified by various clinical manifestations such as anti-SSA/B antibodies, anti-Sm antibodies, anti-RNP antibodies, anti-dsDNA antibodies, low C3 or C4 levels, nephritis, neurological disorder, arthritis, haematologic disorder, rash and SLE/pSS. In addition, the rs485497 variants significantly associated with nephritis and low C3 or C4 levels in Han Chinese. No evidence was found of IL12A SNPs being closely associated with SLE.

GTF2I is a regulator of transcription and acts through direct binding to DNA. Studies suggest that the GTF2I gene is important in the aetiology of autism in individuals with social interaction problems (27) and considered to be one of the main genes responsible for neurocognitive defects in Williams-Beuren syndrome (28). TFII-I, encoded by the GTF2I gene, is a multifunctional protein with the role in transcriptional regulation of critical developmental genes that control cell proliferation (c-FOS), cell cycle (cyclin D1) and developmental processes (29). It binds specifically to several DNA sequence elements and mediates growth factor signalling (30). It has a role in the PI3K/AKT signalling pathway (31), which plays a key role in diverse physiologic processes, including dendritic spine formation during development and structural synaptic plasticity (32).

Table IV. Frequencies of 11 SNPs genotypes with various clinical features.

Subphenotypes	Comparison	rs117026326(GTF2I)		rs4717901(GTF2IRD1)		rs485497(IL12A)		rs583911(IL12A)	
		p	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)
Anti- SSA*	P (n=454) vs N (n=469)	0.46	1.07(0.89-1.30)	0.79	0.97(0.81-1.18)	0.54	1.07(0.86-1.32)	0.39	1.09(0.89-1.33)
	P (n=454) vs C (n=938)	3.68×10^{-43}	3.52(2.93-4.24)	1.59×10^{-17}	2.14(1.79-2.55)	0.37	0.92(0.77-1.10)	0.81	0.98(0.82-1.16)
	N (n=469) vs C (n=938)	9.70×10^{-39}	3.29(2.73-3.95)	4.91×10^{-19}	2.19(1.84-2.61)	0.11	0.86(0.72-1.03)	0.21	0.90(0.75-1.07)
Anti-SSB*	P (n=105) vs N (n=818)	0.16	0.80(0.59-1.09)	0.027	0.70(0.51-0.96)	0.08	1.33(0.97-1.83)	0.36	1.15(0.85-1.57)
	P (n=105) vs C (n=938)	3.4×10^{-11}	2.80(2.05-3.84)	0.005	1.58(1.15-2.18)	0.4	1.14(0.83-1.57)	0.70	1.06(0.78-1.44)
	N (n=818) vs C (n=938)	1.34×10^{-54}	3.48(2.96-4.100	1.98×10^{-26}	2.25(1.94-2.62)	0.053	0.86(0.74-1.00)	0.26	0.92(0.80-1.06)
Anti-Sm*	P (n=184) vs N (n=739)	0.727	0.96(0.76-1.21)	0.29	0.88(0.70-1.12)	0.75	0.96(0.74-1.24)	0.50	0.92(0.72-1.17)
	P (n=184) vs C (n=938)	2.28×10^{-23}	3.38(2.64-4.34)	2.96×10^{-8}	1.99(1.56-2.55)	0.19	0.84(0.64-1.09)	0.19	0.84(0.66-1.09)
	N (n=739) vs C (n=938)	1.34×10^{-47}	3.33(2.81-3.93)	5.80×10^{-22}	2.15(1.83-2.51)	0.18	0.90(0.77-1.05)	0.96	0.99(0.82-1.11)
Anti-RNP*	P (n=270) vs N (n=653)	0.55	1.07(0.86-1.32)	0.81	0.97(0.79-1.20)	0.87	0.98(0.77-1.24)	0.82	1.03(0.82-1.28)
	P (n=270) vs C (n=938)	2.65×10^{-31}	3.5(2.81-4.34)	1.13×10^{-11}	2.07(1.68-2.57)	0.24	0.87(0.70-1.09)	0.64	0.95(0.77-1.17)
	N (n=653) vs C (n=938)	4.99×10^{-44}	3.37(2.76-3.89)	2.13×10^{-20}	2.13(1.81-2.50)	0.17	0.76(0.76-1.05)	0.33	0.93(0.79-1.08)
Anti-dsDNA*	P (n=445) vs N (n=503)	0.45	1.08(0.89-1.31)	0.98	1.00(0.82-1.22)	0.72	1.04(0.84-1.29)	0.85	0.98(0.80-1.20)
	P (n=445) vs C (n=938)	2.07×10^{-40}	3.47(2.87-4.19)	2.54×10^{-16}	2.12(1.77-2.54)	0.29	0.90(0.75-1.09)	0.38	0.92(0.77-1.10)
	N (n=503) vs C (n=938)	2.50×10^{-37}	3.22(2.68-3.87)	3.37×10^{-17}	2.11(1.77-2.52)	0.13	0.87(0.72-1.04)	0.49	0.94(0.79-1.12)
Low C3 or C4 levels	P (n=591) vs N (n=357)	0.79	0.97(0.80-1.19)	0.31	1.11(0.65-1.02)	0.07	0.81(0.65-1.02)	0.11	0.84(0.68-1.04)
	P (n=591) vs C (n=938)	8.62×10^{-43}	3.30(2.77-3.94)	5.67×10^{-17}	2.19(1.86-2.59)	0.02	0.82(0.69-0.97)	0.11	0.88(0.74-1.03)
	N (n=357) vs C (n=938)	9.70×10^{-39}	3.29(2.73-3.95)	4.91×10^{-19}	2.19(1.84-2.61)	0.11	0.86(0.72-1.03)	0.21	0.90(0.75-1.07)
Nephritis	P (n=517) vs N (n=431)	0.039	0.82(0.68-0.99)	0.41	0.92(0.76-1.12)	0.11	0.84(0.68-1.04)	0.28	0.90(0.74-1.09)
	P (n=517) vs C (n=938)	2.84×10^{-36}	3.09(2.58-3.70)	2.13×10^{-17}	2.08(1.75-2.47)	0.03	0.82(0.69-0.98)	0.17	(0.76-1.05)
	N (n=431) vs C (n=938)	5.63×10^{-47}	3.77(3.12-4.54)	2.10×10^{-19}	2.25(1.88-2.69)	0.79	0.98(0.81-1.17)	0.96	0.99(0.84-1.19)
Neuropsychiatric disorder	P (n=138) vs N (n=810)	0.96	1.01(0.77-1.31)	0.54	1.09(0.83-1.42)	0.46	0.89(0.66-1.21)	0.66	0.94(0.71-1.25)
	P (n=138) vs C (n=938)	8.07×10^{-20}	3.4(2.59-4.49)	4.43×10^{-10}	2.32(1.77-3.03)	0.16	0.81(0.60-1.09)	0.41	0.89(0.67-1.18)
	N (n=810) vs C (n=938)	1.14×10^{-51}	3.38(2.88-3.98)	7.27×10^{-23}	2.13(1.83-2.48)	0.20	0.91(0.78-1.05)	0.46	0.95(0.82-1.09)
Arthritis	P (n=381) vs N (n=567)	0.74	0.97(0.80-1.17)	0.13	0.86(0.71-1.05)	0.92	1.01(0.82-1.25)	0.60	1.06(0.86-1.29)
	P (n=381) vs C (n=938)	4.09×10^{-35}	3.33(2.73-4.05)	1.86×10^{-12}	1.97(1.63-2.38)	0.31	0.90(0.74-1.10)	0.79	0.98(0.81-1.17)
	N (n=567) vs C (n=938)	7.35×10^{-46}	3.43(2.88-4.09)	3.86×10^{-23}	2.28(1.94-2.69)	0.19	0.89(0.75-1.06)	0.33	0.92(0.79-1.09)
Haematologic disorder	P (n=305) vs N (n=643)	0.17	1.15(0.94-1.40)	0.50	1.07(0.88-1.31)	0.61	1.94(0.75-1.18)	0.24	0.88(0.71-1.09)
	P (n=305) vs C (n=938)	6.69×10^{-38}	3.72(3.03-4.59)	7.76×10^{-16}	2.26(1.85-2.77)	0.15	0.85(0.69-1.06)	0.14	0.86(0.70-1.05)
	N (n=643) vs C (n=938)	9.86×10^{-44}	3.24(2.73-3.84)	3.43×10^{-20}	2.11(1.80-2.48)	0.23	0.91(0.77-1.07)	0.73	0.97(0.83-1.14)
Rash	P (n=347) vs N (n=601)	0.45	1.08(0.89-1.31)	0.81	0.98(0.80-1.19)	0.64	0.95(0.76-1.18)	0.46	0.92(0.75-1.14)
	P (n=347) vs C (n=938)	2.03×10^{-37}	3.55(2.90-4.34)	2.22×10^{-14}	2.11(1.74-2.57)	0.16	0.86(0.70-1.06)	0.27	0.90(0.74-1.09)
	N (n=601) vs C (n=938)	2.56×10^{-43}	3.29(2.77-3.92)	1.06×10^{-20}	2.17(1.84-2.55)	0.27	0.91(0.77-1.08)	0.70	0.97(0.83-1.14)
SLE/SS	P (n=84) vs N (n=864)	0.43	1.14(0.82-1.58)	0.98	0.99(0.71-1.40)	0.60	1.11(0.77-1.60)	0.94	1.01(0.71-1.44)
	P (n=84) vs C (n=938)	4.60×10^{-16}	3.76(2.68-5.27)	1.30×10^{-5}	2.10(1.50-2.96)	0.87	0.97(0.68-1.39)	0.75	0.95(0.67-1.34)
	N (n=864) vs C (n=938)	2.82×10^{-49}	3.3(2.8-3.88)	2.33×10^{-22}	2.11(1.82-2.46)	0.09	0.88(0.75-1.02)	0.34	0.93(0.81-1.08)

P: patients positive for a certain phenotype; N: patients negative for a certain phenotype; C: controls. *Data were available for 923 patients; Bonferroni corrections data not shown.

GTF2IRD1 is a prime candidate for some of the major features of Williams-Beuren syndrome, presumably caused by abnormally reduced abundance of this putative transcriptional repressor protein (33). Prior studies demonstrated a role for GTF2IRD1 in the motoric and anxiety-related abnormalities in Williams-Beuren syndrome, and suggested basal ganglia and potentially cerebellar abnormalities in GTF2IRD1 mice (34). GTF2I and GTF2IRD1 contribute to the development of neural pathways involved in visual spatial cognition and in human neurodevelopment and cognition (35). pSS patients commonly experience fatigue, pain and cognitive symptoms. The prevalence of depression in pSS is between 30 to 50% (36, 37). Besides, Neuropsychiatric systemic lupus erythematosus (NPSLE) may present in approximately one-half of patients with SLE. Autoantibody mediated vascular or neuronal injury seems to play a major role in NPSLE. But the underlying mechanisms of NPSLE are not clear. Our data showed that there was no significant difference between NPSLE and negative ones. Further study would help us clarify if there were any association between neuropsychiatric disorder and GTF2I, GTF2IRD1 in both pSS and SLE patients. Anti-SSA/B antibodies were special antibodies of both SLE and pSS. SNPs rs117026326 of GTF2I and rs4717901of GTF2IRD1 were associated with SLE patients with or without anti-SSA/B antibodies and there were no significant difference between anti-SSA/B antibodies positive and negative patients. Moreover, the same results were found in SLE/SS subphenotype. Considering both pSS and SLE have strong association with GTF2I, GTF2IRD1, these genes may be shared with other autoimmune diseases. More detailed studies are required to determine which molecular mechanisms are controlled by GTF2I and GTF2IRD1 genetic variants. IL-12, a pro-inflammatory cytokine, was produced by Dendritic cells, macrophages and B cells (38), which is a central cytokine in pSS pathogenesis, promoting activation of the type II IFN system via both the innate (natural killer cells) and the adaptive (type 1 T-helper cells) immune systems (39). IL12A acts with the IL12B subunit to signal as a dimer through STAT4 to induce the differentiation of naive $CD4^+$ T cells into type 1 T-helper cells, thereby causing these

cells to produce IFN-γ (40). Multiple SNPs from various genes (rs485497 and rs583911) have been reported to relate to various diseases, such as pSS, PBC, Graves' disease and tumour (41-43). Our results indicate that there are no associations of rs485497 and rs583911 with SLE, but we rather revealed the association of these rs485497 with nephritis and low C3 or C4 levels of SLE in Chinese Han. These findings suggest that the rs485497 and rs583911 variants may be a contributing future that underlie a wide spectrum of autoimmune diseases, but not to SLE in particular. Furthermore, fundamental differences may exist in the IL12A related genetic pathogeneses of SLE and other autoimmune diseases. Nevertheless, it is likely that difference in genetic background between SLE and other autoimmune diseases, as well as the potential genetic heterogeneity among different populations, may account for these variations.

In summary, our data indicated that the GTF2I region (rs117026326), the GTF2IRD1 region (rs4717901), but not the IL12A region (rs485497, rs583911), were associated with the development of SLE in Chinese Han. A larger sample size and more SNPs might be required for further analysis of GTF2I, GTF2IRD1 and IL12A gene with SLE susceptibility in different ethnicities.

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