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IL12B and *IL17* genes polymorphisms associated with differential susceptibility to juvenile idiopathic arthritis and juvenile-onset systemic lupus erythematosus in Chinese children

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Abstract

Genetic factors play a crucial role in the immune response of juvenile idiopathic arthritis (JIA) and juvenile-onset systemic lupus erythematosus (JSLE). This study aimed to investigate the association of *IL12B* (rs3212227, rs6887695) and *IL17* (rs2275913, rs763780) gene polymorphisms with the susceptibility of JIA and JSLE in Chinese children. A total of 303 healthy controls and 304 patients including 160 JIA and 144 patients were analyzed, and the genetic polymorphisms were genotyped by using a Sequenom MassArray system. There was a significant association between the *IL12B* rs3212227 genotype and the increased risk of JSLE (P = .01). For rs6887695, the minor allele C was significantly associated with the increased risk of JIA (odds ratio = 1.48, 95% confidence interval [CI] = 1.12–1.95, P = .005). Moreover, rs6887695 genotype was significantly associated with both JIA and JSLE susceptibility (P < .05). Besides, *IL12B* haplotype GC significantly associated with the increased risk of JIA (P = .016). However, no significant difference was found between the *IL17* (rs2275913, rs763780) gene polymorphisms and JIA or JSLE susceptibility (P > .05). And similar genotype distributions of *IL12B* and *IL17* polymorphisms were found between the patients with nephritis and without nephritis in JSLE (P > .05). Our results indicated that *IL12B* polymorphisms was associated with an increased risk for the development of JIA and JSLE in Chinese children, highlighting the involvement of inflammation in the pathogenesis of JIA and JSLE. Moreover, there was a risk haplotype in *IL12B* which could increase the risk of JIA.

Abbreviations: AS = ankylosing spondylitis, CI = confidence interval, IL-12B = interleukin 12B, IL-17 = interleukin 17, JIA = juvenile idiopathic arthritis, JSLE = juvenile systemic lupus erythematosus, OR = odds ratio.

Keywords: gene polymorphism, IL12B, IL17, juvenile idiopathic arthritis, juvenile-onset systemic lupus erythematosus

1. Introduction

Juvenile idiopathic arthritis (JIA) and juvenile-onset systemic lupus erythematosus (JSLE) are 2 common complex autoimmune diseases in children. JIA is the most common chronic inflammatory arthropathy occurred in children below 16 years, featured on chronic joint synovitis and extra articular organs' damages.[1] JSLE is another systemic autoimmune disorder with disease-onset before the age of 18, which accounts for up to 20% of all SLE patients.[2] Compared to SLE in adults, juvenile-onset SLE is accompanied by more severe multi-organ involvement, higher disease activity, earlier development of damage, and increased use of immunosuppressive treatment.[3,4] Considering the insufficient therapeutic options and more severe damage, a greater understanding of pathogenesis is required.

The pathogenesis of JIA and JSLE is unclear, but genetic factors, immune mechanisms, and environmental exposures are presumed to be interactively involved in the continuous autoimmune process.[5–7] Genes encoding cytokines, determine the course of T-cell-mediated immune response, play an undisputedly critical role in the development of the diseases.[8] Interleukin-12 (IL-12) is a pro-inflammatory heterodimeric cytokine, which is primarily generated by different antigen presenting cells that induces the production of interferon-γ (IFN-γ) and it is a key modulator

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Medical Ethics Committee of West China Second University Hospital of Sichuan University (protocol 2021176).

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of innate and adaptive immunity.[9,10] IL-12 is comprised of 2 subunits, IL-12p35 encoded by the IL-12A gene and IL-12p40 encoded by the interleukin 12B (IL-12B) gene.[11] A few polymorphisms in the *IL12B* has been identified, especially the *IL12B* gene 3' untranslated region (UTR) polymorphism. In addition, some studies have found the significant association between *IL12B* polymorphisms and the susceptibility to autoimmune diseases, such as rheumatoid arthritis (RA),[12–14] adult SLE,[15–17] ankylosing spondylitis (AS)[18] and type-1 diabetes (T1D).[19,20] However, the impact of rs3212227 and rs6887695 in *IL12B* on JIA and JSLE risk and severity has not yet reported in Chinese children. Thus, we investigate the correlation of the 2 SNPs in *IL12B* with the susceptibility to JIA and JSLE in this study.

Furthermore, IL-12p40 subunit also comprises pro-inflammatory cytokine IL-23 with p19 subunit, which is an important regulator of T-helper-17 cells (Th17) cells. Th17 cells preferentially produce interleukin 17 (IL) IL-17A, IL-17F, IL-21, and IL-22.[21] IL-17A and IL-17F are 2 important members in the IL-17 family, they share up to 60% structure homology and induce the production of inflammatory mediators.[21] To date, the SNPs in the *IL17* gene have been reported to be associated with an increased risk for different autoimmune diseases including RA,[22,23] AS,[24,25] spondyloarthritis,[26] inflammatory bowel disease[27] and adult SLE.[28,29] However, few studies were performed to investigate the role of *IL17* SNPs in JIA and JSLE in a Chinese population. Hence, the influence of *IL17A* rs2275913 and *IL17F* rs763780 on JIA and JSLE were studied in our study.

In the present study, we analyzed the potential associations of the 4 SNPs in *IL12B* and *IL17* genes with the risk and clinical characteristics of JIA and JSLE in Southwest Chinese Han pediatric population. In addition, we investigated the association between the *IL12B* haplotypes and the susceptibility to JIA and JSLE. This is the first study directed at the Chinese Han pediatric population.

2. Materials and methods

2.1. Study subjects

The study group consisted of 607 subjects, including 160 JIA patients, 144 JSLE patients and 303 healthy controls. All the subjects were recruited from West China Second University Hospital from November 2017 to August 2021. JIA patients were diagnosed according to the International League of Associations for Rheumatology criteria.[30] JSLE was diagnosed according to the revised American College of Rheumatology criteria for the diagnosis of SLE before the age of 18 years.[31] Members of the control group, who had no inter-current infections, inflammatory diseases, or had undergone elective surgery, were chosen from the Health Examination Center of West China Second University Hospital. The study was approved by the Institutional Ethics Committee of West China Second University Hospital of Sichuan University and complied with the Declaration of Helsinki. Written informed consent was obtained prior to enrollment from all subjects or their parents/guardians.

2.2. Measurement of clinical parameter

Blood samples were collected at the fasting state from each subject. Serum Vitamin D was detected by chemoluminescence (ARCHITECT i2000, Abbott). Erythrocyte sedimentation rate (ESR) was measured by an auto-ESR meter (Alifax Test 1, Alifax, Italy). HLA-B27 was detected by flow cytometry (Canto II, Beckman Coulter). Serum anti-nuclear antibodies and anti-citrullinated protein antibodies were detected by immunofluorescence and enzyme-linked immunosorbent assay respectively (EUROIMMUN, Germany). Serum rheumatoid factor, Complement C3 and Complement C4 were measured by nephelometry (BN II, SIEMENS, Germany).

2.3. IL12B and IL17 polymorphisms

Genomic DNA was isolated from peripheral blood samples by using the Genomic DNA kit (Tiangen; Beijing, China), and the concentration was measured by a NanoDrop 2000c spectrophometer (Thermo Scientific, DE). The extracted DNA was detected immediately or stored at −80°C for <6 months.

The information of SNPs in *IL12B* and *IL17* genes were shown in Supplementary Table 1, http://links.lww.com/MD/J369. Primers for the 4 SNPs were shown in Supplementary Table 2, http://links.lww.com/MD/J370. The SNPs were genotyped using a Sequenom MassArray system (iPLEX assay, Sequenom) according to the manufacturer instructions. The DNA samples were amplified by multiplex polymerase chain reaction (PCR), and the products were used for locus-specific single-base extension reactions. The final products were desalted and transferred to 384-element SpectroCHIP arrays (Sequenom). Allele detection was accomplished by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). The resultant mass spectrograms and genotype data were analyzed by the MassArray Typer 4.0 software (Supplementary Fig. 1, http://links.lww.com/MD/J371).

2.4. Statistical analysis

The clinical characteristics of the participants were expressed as mean ± standard deviation for normally distributed variables. Continuous variables with a skewed distribution were described with the median and interquartile. Hardy–Weinberg equilibrium was independently appraised for each polymorphism. The effect of SNPs was tested for odds ratio (OR), 95% confidence interval (CI), and *P* values; all the tests were adjusted for age and gender by binary logistic regression.

When comparing the 2 groups of subjects (case and control), the following association analytical methods were used: comparing allelic frequencies (major allele "A" vs minor allele "B"); dominant gene model (AA vs AB + BB); recessive gene model (AA + AB vs BB); and additive gene model (AA vs AB vs BB). Haplotype analysis was also performed, considering that it can also capture additional significant variants since it more sensitive than single SNP analysis. Haplotypes with frequencies of more than 0.03 were analyzed. The haplotype frequency calculation was performed using HaploView 4.2 (Whitehead Institute for Biomedical Research, MIT Media Lab, and Broad Institute of Harvard and MIT).[32]

All the statistical analyses were performed using SPSS 21.0 software (IBM Corporation, New York) and SHEsis online software (http://shesisplus.bio-x.cn/SHEsis.html).[33] Additional information on statistical methods is available in the supplementary material. A 2-sided *P* value of <.05 was deemed statistically significant.

3. Results

3.1. Demographic and clinical characteristics

Demographic and clinical characteristics of the study subjects are described in Table 1. All the study participants were Chinese children. The mean age in healthy controls, JIA patients and JSLE patients was 9.47 years, 9.97 years, and 12.24 years old, respectively. The average disease duration of JIA patients and JSLE patients was 12.00 months and 21.96 months. Among all the JIA patients, the prevalence of ANA, RF, ACPA, and HLA-B27 was 14.38%, 15.00%, 11.25%, and 18.13% respectively; the proportions of systemic disease, polyarthritis, and oligoarthritis was 21.2%, 23.8%, and 20%, respectively. Among all the JSLE patients, the positive frequencies of ANA and lupus nephritis was 90.97% and 44.44%, respectively.

3.2. Association of IL-12B and IL-17 polymorphisms with the risk of JIA and JSLE

We genotyped 4 SNPs (*IL12B* rs3212227, *IL12B* rs6887695, *IL17A* rs2275913, and *IL17F* rs763780) in 303 healthy controls and 304 patients, including 160 JIA patients and 144 JSLE patients. The distributions of the allele frequencies for the 4 SNPs complied with Hardy–Weinberg equilibrium in the controls among the Chinese Han population ($P > .05$) (Tables 2 and 3). As shown, the rs3212227 genotype was significantly associated with JSLE susceptibility {dominant model, OR = 2.05, 95% CI = 1.21–3.48, $P = .01$; additive model (TG vs TT): OR = 2.27, 95% CI = 1.30–3.96, $P = .01$} (Table 2). Nonetheless, no trend for significant association was observed between the rs3212227 genotype and JIA (Table 2).

For rs6887695, we found that there was a significant association between the *IL12B* rs6887695 G/C variant and JIA patients, with minor allele C associated with the risk of JIA (allele frequency, OR = 1.48, 95% CI = 1.12–1.95, $P = .005$) (Table 2). Moreover, we found that rs6887695 genotype was significantly associated with JIA susceptibility {dominant model, OR = 1.83, 95% CI = 1.19–2.79, $P = .005$; additive model (GC vs GG): OR = 1.70, 95% CI = 1.09–2.67, $P = .02$; additive model (CC vs GG): OR = 2.14, 95% CI = 1.19–3.85, $P = .01$} (Table 2). Besides, significant association was also observed between the rs6887695 genotype and JSLE susceptibility {dominant model, OR = 1.74, 95% CI = 1.05–2.90, $P = .03$; additive model (GC vs GG): OR = 1.87, 95% CI = 1.09–3.20, $P = .02$} (Table 2).

However, no significant difference was observed between JIA patients and healthy controls in the allele frequency of *IL17A* rs2275913 and *IL17F* rs763780 (rs2275913, OR = 1.05, 95% CI = 0.80–1.38, $P = .73$; rs763780, OR = 1.06,

Table 1

Demographic and clinical characteristics of the study participants.

Variables	Healthy controls	JIA	JSLE
Number of subjects	303	160	144
Age, yr, mean ± SD†	9.47 ± 3.68	9.97 ± 3.58	12.24 ± 2.56
Sex (male/female)	170/133	89/72	16/129
Disease duration (mo)‡	-	12.00 (6.00–36.00)	21.96 (6.96–38.04)
Vitamin D (ng/mL)‡	17.60 (12.98–24.80)	24.30 (18.58–28.58)	21.30 (15.65–28.05)
ANA (N [%])	-	23 (14.38%)	131 (90.97%)
Lupus nephritis (N [%])	-	-	64 (44.44%)
Rheumatoid factor (N [%])	-	24 (15%)	-
ACPA positive (N [%])	-	18 (11.25%)	-
HLA-B27 (N [%])	-	29 (18.13%)	
Systemic disease/Polyarthritis/Oligoarthritis (N [%])	-	34 (21.2%)/38 (23.8%)/32 (20%)	-
ESR (mm/h)‡	-	13.00 (2.00–27.50)	9.00 (4.00–18.00)
Serum complement C3 (g/L)‡	-	1.08 (0.94–1.35)	0.74 (0.62–0.90)
Serum complement C4 (g/L)‡	-	0.22 (0.18–0.29)	0.14 (0.10–0.18)

ACPA = anti-citrullinated protein antibodies, ANA = anti-nuclear antibodies, ESR = erythrocyte sedimentation rate, HLA-B27 = human leukocyte antigen B27, JIA = juvenile idiopathic arthritis, JSLE = juvenile-onset systemic lupus erythematosus, SD = standard deviation.

†Data with normal distribution expressed as arithmetic mean ± standard deviation (SD).

‡Data with skew distribution were expressed as median (interquartile range).

Table 2

Genotype and allele analyses of the *IL12B* gene SNPs in patients with JIA, JSLE and healthy controls.

					Controls vs JIA		Controls vs JSLE	
SNPs and nucleotides	Genotype/ allele	Controls†, n (%)	JIA, n (%)	cSLE, n (%)	Age- and gender-adjusted OR (95% CI)‡	P‡	Age- and gender-adjusted OR (95% CI)‡	P‡
rs3212227	TT	105 (35.1)	44 (27.5)	37 (25.7)	1.00 (ref.)		1.00 (ref.)	
(T > G)	TG	137 (45.8)	86 (53.8)	83 (57.6)	1.52 (0.97–2.38)	.07	**2.27 (1.30–3.96)**	**.01**
	GG	57 (19.1)	30 (18.8)	24 (16.7)	1.25 (0.71–2.21)	.44	1.52 (0.74–3.15)	.26
	TG + GG	194 (64.9)	116 (72.5)	107 (74.3)	1.46 (0.96–2.22)	.08	**2.05 (1.21–3.48)**	**.01**
	TG + TT	242 (80.9)	130 (81.2)	120 (83.3)	1.00 (ref.)		1.00 (ref.)	
	GG	57 (19.1)	30 (18.8)	24 (16.7)	0.96 (0.59–1.58)	.88	0.92 (0.49–1.73)	.79
	T	347 (58.0)	174 (54.4)	157 (54.5)	1.00 (ref.)		1.00 (ref.)	
	G	251 (42.0)	146 (45.6)	131 (45.5)	1.16 (0.88–1.53)	.28	1.33 (0.95–1.88)	.10
	HWE	$P = .59$						
rs6887695	GG	116 (38.9)	42 (26.3)	44 (30.5)	1.00 (ref.)		1.00 (ref.)	
(G > C)	GC	140 (47.0)	85 (53.1)	79 (54.9)	**1.70 (1.09–2.67)**	**.02**	**1.87 (1.09–3.20)**	**.02**
	CC	42 (14.1)	33 (20.6)	21 (14.6)	**2.14 (1.19–3.85)**	**.01**	1.41 (0.66–3.00)	.37
	GC + CC	182 (61.1)	118 (73.7)	100 (69.5)	**1.83 (1.19–2.79)**	**.005**	**1.74 (1.05–2.90)**	**.03**
	GC + GG	256 (85.9)	127 (79.4)	123 (85.4)	1.00 (ref.)		1.00 (ref.)	
	CC	42 (14.1)	33 (20.6)	21 (14.6)	1.53 (0.92–2.54)	.10	0.96 (0.48–1.92)	.91
	G	372 (62.4)	169 (52.8)	167 (58.0)	1.00 (ref.)		1.00 (ref.)	
	C	224 (37.6)	151 (47.2)	121 (42.0)	**1.48 (1.12–1.95)**	**.005**	1.29 (0.91–1.82)	.15
	HWE	$P = .99$						

Bold values are statistically significant ($P < .05$).

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odds ratio.

†Missing data were excluded from the analyses for HC (rs3212227, n = 4; and rs6887695, n = 5).

‡Logistic regression models were used to calculate odds ratios (ORs) and 95% CIs.

95% CI = 0.72–1.56, *P* = .78) (Table 3). Meanwhile, there was no significant association between the 2 SNPs and the JSLE susceptibility (rs2275913, OR = 1.22, 95% CI = 0.87–1.72, *P* = .24; rs763780, OR = 1.26, 95% CI = 0.79–2.01, *P* = .33) (Table 3).

3.3. Correlation of IL-12B and IL-17 polymorphisms with nephritis of JSLE patients

In the present study, the prognostic role that might be played by rs3212227, rs6887695, rs2275913, and rs763780 in JSLE was examined (Table 4). Therefore, subgroup analysis was performed in JSLE patients with nephritis and without nephritis. Similar genotype distributions of *IL12B* and *IL17* polymorphisms were found between the 2 groups {dominant model: rs3212227, OR = 0.81, 95% CI = 0.38–1.72, *P* = .58; rs6887695, OR = 1.05, 95% CI = 0.51–2.15, *P* = .91; rs2275913, OR = 0.59, 95% CI = 0.28–1.24, *P* = .16; rs763780, OR = 1.51, 95% CI = 0.74–3.09, *P* = .26} (Table 4).

3.4. Haplotype analysis of IL-12B with the risk of JIA and JSLE

Haplotypes were constructed upon the basis of 2 IL12B SNPs (rs3212227 and rs6887695). Then we investigated whether any specific haplotype would confer a higher risk or protection for JIA or JSLE. Analysis of the haplotype structure revealed 4 distinct haplotypes in JIA and 4 haplotypes in JSLE patients (Tables 5 and 6). We observed that IL12B haplotype GC was associated with an increased risk of JIA (OR = 1.419, 95% CI = 1.064–1.892, *P* = .016) (Table 5). However, no significant haplotype differences were observed between JSLE patients and healthy controls (*P* > .05) (Table 6).

4. Discussion

JIA and JSLE are systemic autoimmune diseases with the involvement of the immune system and systemic manifestations. Both of them show some clinical similarities, including fever, arthralgia, arthritis and the presence of autoantibodies. Given these common features, it is not surprising that they can also share part of the same disease mechanisms and susceptibility loci. Although the pathogenesis of the 2 diseases is complex and ambiguous, the genes polymorphisms increase our understanding of the mechanisms. And their prompt diagnosis is important to start the appropriate treatment, reduce complications and improve the life quality in most families.

The risk variants of the diseases are often population specific, although much evidence showed that disease etiology is common

Table 3

Genotype and allele analyses of the *IL17* gene SNPs in patients with JIA, JSLE and healthy controls.

					Controls vesus JIA		Controls vesus JSLE	
SNPs and nucleotides	Genotype/ allele	Controls†, n (%)	JIA, n (%)	cSLE, n (%)	Age- and gender-adjusted OR (95% CI)‡	P‡	Age- and gender-adjusted OR (95% CI)‡	P‡
rs2275913	GG	89 (29.8)	42 (26.2)	39 (27.1)	1.00 (ref.)		1.00 (ref.)	
(G > A)	GA	150 (50.2)	88 (55.0)	68 (47.2)	1.24 (0.79–1.95)	.35	1.09 (0.62–1.90)	.77
	AA	60 (20.0)	30 (18.8)	37 (25.7)	1.08 (0.60–1.92)	.81	1.53 (0.77–3.06)	.23
	GA + AA	210 (70.2)	118 (73.8)	105 (72.9)	1.19 (0.77–1.84)	.43	1.21 (0.71–2.05)	.50
	GA + GG	239 (80.0)	130 (81.2)	107 (74.3)	1.00 (ref.)		1.00 (ref.)	
	AA	60 (20.0)	30 (18.8)	37 (25.7)	0.93 (0.57–1.52)	.78	1.44 (0.81–2.56)	.21
	G	328 (54.8)	172 (53.8)	146 (50.7)	1.00 (ref.)		1.00 (ref.)	
	A	270 (45.2)	148 (46.2)	142 (49.3)	1.05 (0.80–1.38)	.73	1.22 (0.87–1.72)	.24
	HWE	*P* = .98						
rs763780	TT	221 (73.7)	119 (74.4)	99 (68.8)	1.00 (ref.)		1.00 (ref.)	
(T > C)	TC	73 (24.3)	35 (21.9)	40 (27.8)	0.91 (0.57–1.44)	.68	1.31 (0.76–2.25)	.34
	CC	6 (2.0)	6 (3.7)	5 (3.4)	1.86 (0.59–5.92)	.29	1.34 (0.28–6.42)	.72
	TC + CC	79 (26.3)	41 (25.6)	45 (31.2)	0.98 (0.63–1.52)	.93	1.31 (0.77–2.22)	.31
	TC + TT	294 (98.0)	154 (96.3)	139 (96.6)	1.00 (ref.)		1.00 (ref.)	
	CC	6 (2.0)	6 (3.7)	5 (3.4)	1.91 (0.60–6.03)	.27	1.27 (2.27–5.98)	.77
	T	515 (85.8)	273 (85.3)	238 (82.6)	1.00 (ref.)		1.00 (ref.)	
	C	85 (14.2)	47 (14.7)	50 (17.4)	1.06 (0.72–1.56)	.78	1.26 (0.79–2.01)	.33
	HWE	*P* = .99						

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, JSLE = juvenile systemic lupus erythematosus, OR = odds ratio.

†Missing data were excluded from the analyses for HC (rs2275913, n = 4; and rs763780, n = 3).

‡Logistic regression models were used to calculate odds ratios (ORs) and 95% CIs.

Table 4

Correlation of *IL12B* and *IL17* polymorphisms with nephritis in JSLE patients.

Genes	SNPs	Genotype	Without nephritis, *n* (%)	With nephritis, *n* (%)	Age- and gender-adjusted OR (95% CI)†	*P*†
IL12B	rs3212227	TT	19 (23.7)	18 (28.1)	1.00 (ref.)	
		TG + GG	61 (76.3)	46 (71.9)	0.81 (0.38–1.72)	.58
IL12B	rs6887695	GG	25 (31.2)	19 (29.7)	1.00 (ref.)	
		GC + CC	55 (68.8)	45 (70.3)	1.05 (0.51–2.15)	.91
IL17A	rs2275913	GG	18 (22.5)	21 (32.8)	1.00 (ref.)	
		GA + AA	62 (77.5)	43 (67.2)	0.59 (0.28–1.24)	.16
IL17F	rs763780	TT	58 (72.5)	41 (64.1)	1.00 (ref.)	
		CT + CC	22 (27.5)	23 (35.9)	1.51 (0.74–3.09)	.26

CI = confidence interval, JSLE = juvenile systemic lupus erythematosus, OR = odds ratio.

†Logistic regression models were used to calculate odds ratios (ORs) and 95% CIs.

Table 5

Haplotype analysis for genotypes of *IL12B* and JIA risk.

Haplotypes	Case (freq.)	Control (freq.)	OR (95%CI)	*P*
TG	140 (0.437)	296 (0.503)	0.814 (0.62–1.069)	.139
GG	29 (0.09)	68 (0.115)	0.788 (0.498–1.245)	.307
GC	117 (0.365)	175 (0.297)	**1.419 (1.064–1.892)**	**.016**
TC	34 (0.106)	49 (0.083)	1.351 (0.852–2.14)	.198

Haplotypes with frequency <0.03 are ignored.

Order of IL12B haplotype block: rs3212227 (minor allele G) and rs6887695 (minor allele C).

Bold values are statistically significant ($P < .05$).

CI = confidence interval, OR = odds ratio.

Table 6

Haplotype analysis for genotypes of *IL12B* and JSLE risk.

Haplotypes	Case (freq.)	Control (freq.)	OR (95%CI)	*P*
TG	136 (0.472)	296 (0.503)	0.937 (0.707–1.24)	.650
GG	31 (0.107)	68 (0.115)	0.954 (0.608–1.496)	.838
GC	100 (0.347)	175 (0.297)	1.31 (0.97–1.767)	.076
TC	21 (0.072)	49 (0.083)	0.894 (0.525–1.521)	.679

Haplotypes with frequency <0.03 are ignored.

Order of IL12B haplotype block: rs3212227 (minor allele G) and rs6887695 (minor allele C).

CI = confidence interval, JSLE = juvenile systemic lupus erythematosus, OR = odds ratio.

generally. In this study, we found that the investigated SNPs in *IL12B* gene, but not in *IL17* gene are associated with JIA and JSLE in Chinese children. Specifically, the *IL12B* rs3212227 was associated only with JSLE susceptibility, while *IL12B* rs6887695 was associated with all the 2 diseases. Moreover, we found that *IL12B* haplotype GC significantly associated with JIA susceptibility. To the best of our knowledge, this is the first study demonstrating the associations of *IL12B* polymorphisms and JIA and JSLE in Southwest Chinese children.

These *IL12B* findings, in terms of disease susceptibility, are in line with data in literature and with results obtained in different populations with autoimmune diseases. The *IL12B* rs6887695 polymorphism has been associated with susceptibility to psoriasis,[34] psoriatic arthritis[34] and Crohn disease.[35,36] Moreover, the meta-analysis by J. Li, et al suggested that *IL12B* rs3212227 polymorphism might be associated with genetic susceptibility to T1D, RA, Behcet disease, but not Graves' disease and AS in East Asians.[37] In contrast, A. Paradowska-Gorycka, et al reported that genotype and allele frequencies of the *IL12B* rs3212227 variants were not significantly different between SLE patients and controls in a Polish population.[17] The *IL12B* rs3212227 polymorphism was associated with the mean value of the platelets, urea and complement C3 level.[17] Discrepancies in genotype frequencies between the reports may be explained by the heterogeneity of the analyzed diseases and distinct ethnicities as well as limited information in the subgroups.

IL-12, a critical pro-inflammatory cytokine, plays a central role in promoting the differentiation of naive CD4 + T cells into mature IFN-γproducing T-helper (Th1) effect cells. Besides, it is a potent stimulus of natural killer (NK) and CD8 + T cells to produce IFN-γ.[9] *IL12B* is the gene that encodes the p40 subunit of IL-12 and IL-23, thus is involved in both the IL12/Th1 pathway and IL23/Th17 pathway, which play a fundamental role in autoimmunity.[10] Some previous studies demonstrated that IL-12 level in serum was significantly higher in RA patients compared with controls, and also in severe disease phenotypes.[12,14] Moreover, circulating IL-23 and IL-12p40 were raised among AS patients.[18] These evidences support the hypothesis that *IL12B* polymorphisms may impact the autoimmune diseases by several ways: influencing the gene transcription, regulating the expression of cytokines, changing the binding with IL-12 receptor (IL-12R) and subsequently lead to defective IL-12-induced signaling. The identification of risk alleles and their increased abundance in patients with JIA and JSLE promise potential for future patient stratification and the introduction of individualized, target-directed treatment options.

IL-17 is also an important pro-inflammatory cytokine in autoimmune diseases, which plays a vital role in the development of tissue inflammation and organ damage. IL-17 family include 6 proteins from IL-17A to IL-17F, which can promote the production of pro-inflammatory cytokines and chemokines.[21] Although the mechanism by which the minor allele of *IL17A* rs2275913 and *IL17F* rs763780 contribute to autoimmune diseases is not well understood, it is thought to influence the IL-17mRNA and modify the protein amino acid sequence, converting the histidine (CAT) to arginine (CGT).[38] Some studies have reported that *IL17* rs763780 C allele was significantly related to RA risk in Caucasians,[23] Mongolians,[36] Brazilians[39] and Pakistanis.[40] And H.F. Pasha, et al also found that there was significant *IL17A* rs2275913 genes polymorphisms with their serum levels, susceptibility, disease activity and severity in Egyptian SLE patients.[28]

However, in our study no significant differences were found between *IL17* polymorphisms and JIA and JSLE susceptibility. Our result is consistent with that of the study in the Egyptian JSLE patients[41] and in the Slavic adult SLE patients.[29] Not only lack of association of the *IL17* polymorphisms with RA susceptibility was reported in case-control studies of Algerian,[22] Polish[42] and Brazilian[39] but also no significant association was observed between *IL17F* gene rs763780 polymorphism and OA risk in Chinese population.[43] The different results among the studies may be attributed to different age onset of the diseases and different populations. To clarify this discrepancy, the correlation between variations in *IL17* SNPs and JIA and JSLE deserves a more thorough sequence analysis, and also the functional roles of these polymorphisms remain to be studied.

Furthermore, the complications of JSLE may also be associated with genetic characteristics.[44] The recent study by H. F. Pasha, et al reported that patients with nephritis who are homozygous for the A allele of rs2275913 in *IL17A* may develop a more severe form of the disease with increased disease activity and disability.[45] However, no associations between

these genotypes and nephritis in JSLE were noted in this study. Considering that limited information in our study made it difficult to stratify analyses between identified genetic loci and the clinical characters, much more sufficient clinical information and larger sample size is required. More attention is needed to be paid to the stratification analysis to investigate the SNP association in different subgroups.

There were several limitations in our research. Firstly, environmental factors differ between the Chinese and other populations, particularly gene-environment interactions were inevitable. Secondly, we did not acquire detailed information about disease severity and medication of the patients, which limited our analyses. Thirdly, we have no information on the IL-12 and IL-17 protein levels in serum, which would be useful for further understanding of the physiological relevance of the gene polymorphisms in the pathogenesis of JIA and JSLE. Finally, the linkage disequilibrium of those loci with other candidate SNPs in the *IL12B* or *IL17* genes was not investigated. Thus, more well-designed studies with more detailed information are needed to elucidate the role of *IL12B* and *IL17* gene polymorphisms in JIA and JSLE.

5. Conclusions

Taken together, this is the first report demonstrating the relationship that *IL12B* rs3212227 and *IL12B* rs6887695 polymorphisms were significantly associated with JSLE susceptibility, and *IL12B* rs6887695 was also strongly associated with JIA susceptibility in Chinese children. Besides, this study revealed that haplotypes in *IL12B* gene are strongly associated with JIA susceptibility, which highlighted the involvement of IL-12B in the pathogenesis of JIA. Further large studies with patients of different ethnic backgrounds as well as functional experiments are warranted to confirm these findings.

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