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PADI4 and IL-33 gene polymorphisms associated with differential susceptibility to juvenile-onset systemic lupus erythematosus and juvenile idiopathic arthritis in Chinese children

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Abstract

Background: Juvenile systemic lupus erythematosus (JSLE) and juvenile idiopathic arthritis (JIA) are two common types of autoimmune diseases in children with unclear pathogenesis. Both peptidyl arginine deiminase type IV (PADI4) and interleukin 33 (IL-33) are the key molecular involved in immune responses in autoimmune diseases. Usually, it may share the same risk genetic alleles for autoimmune diseases.

Methods: So measurement of PADI4 and IL-33 polymorphisms was conducted with 303 healthy controls, 144 JSLE patients and 160 JIA patients in this study.

Results: It demonstrated that there was a significant association between PADI4 genotypes (rs2240340: CT, CT + CC), IL-33 genotype (rs1929992: TT) and JSLE susceptibility in Southwest China population. While no significant association with the risk of JIA were observed no matter at allelic or genotypic levels.

Conclusions: Our study reveals the importance of PADI4 and IL-33 polymorphisms with JSLE risk and their roles in the development of the diseases need more further researches.

Abbreviations: CI = confidence interval, HWE = Hardy–Weinberg equilibrium, IL-33 = Interleukin 33, JIA = juvenile idiopathic arthritis, JSLE = juvenile systemic lupus erythematosus, LN = lupus nephritis, OR = odds ratio, PADI4 = peptidyl arginine deiminase type IV.

Keywords: IL-33, JIA, JSLE, PADI4, polymorphism

1. Introduction

SLE is characterized by disorders and damage to different tissues, various organs, and immune systems associated with disease activity and may develop lupus nephritis (LN). Children with SLE have more severe and complex clinical manifestation including nervous affection when compared with adult patients.[1] JSLE (juvenile systemic lupus erythematosus) patients are diagnosed before 16 years old with an approximate percentage of 15% to 20%.[2] Juvenile idiopathic arthritis (JIA) is a kind of chronic systemic disease with joint swelling and pain and it may cause damage to other tissues and organs. JIA contains 7 types including juvenile idiopathic arthritis-enthesitis-related arthritis. Both JSLE and JIA have the characteristics of a disrupted balance in lymphocyte subgroups.[3] A previous study indicated that S100A8/A9 and S100A12 had a potential promise for the diagnosis of JSLE and active LN except for LN's gold standard: kidney biopsies.[4,5] To date, multiple pathogenic genes including PADI4 (peptidyl arginine deiminase type IV) and Interleukin 33 (IL-33) related to JSLE and JIA have been paid attention to exploring the biomarkers for the diseases.

The expression of the PADI4 gene is related to the development of macrophages and is associated with inflammation and immune response. Arginine residues can be conversed into citrulline by the

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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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enzyme named PADI4.[6] Autoantibodies directed against citrullinated proteins have a possible pathogenic role since they arise early in the development of RA(Rheumatoid arthritis).[6] Genetic variants have potential associations with clinical features of diseases. The distribution of PADI4 alleles and genotypes are different compared with healthy controls in some kinds of autoimmune diseases. It had been studied about the association of PADI4 polymorphisms with RA susceptibility in Egyptians, UK Caucasians, French, Japanese, Korean population, and so on.[7–11] Also, PADI4-19 polymorphism and 2 PADI4 haplotypes(GCC, GCT) reported a significant association with RA in the Egyptian population.[7] Moreover, 3 PADI4 gene polymorphisms (G allele at rs11203366, T allele at rs11203367, and G allele at rs874881) and GTG haplotype were significantly correlated with RA from the Southern México population.[12] The result of PADI4 rs11203367 could fit a meta-analysis of the Asian descent patients.[13] In previous studies, it had a higher frequency for allele C at rs1748033 in the PADI4 gene and its CC genotype with autoimmune thyroid patients, while no significant relationships were found.[14]

But no association between PADI4 rs2240340 polymorphism and RA was found in the Chinese Han population.[15] While few PADI4 gene alleles were involved in JIA susceptibility. So, 3 PADI4 gene SNPs (rs2240340, rs2240337, and rs1748033) were investigated to find whether JIA and RA share the same genetic risk or not. And the study among the Japanese population showed A allele at rs2240337 of the PADI4 gene was significantly associated with ACPA (anti-cyclic citrullinated peptide antibody)-positivity risk in JIA.[16] And recent research showed that the TT genotype of PADI4 rs2240340 was related to JIA significantly.[17]

IL-33 is a cytokine of the IL-1 family members and plays a key role in cancer and autoimmune diseases such as RA, SLE, multiple sclerosis, and diabetes. Some innate immune cells like macrophages and dendritic cells can produce IL-33.[18,19] IL-33 and its receptor ST2 may be involved in pathological processes by inducing Th2-type immune responses.[20] But data about functions and immune mechanisms of IL-33 and ST2 pathways in the development of SLE and JIA are limited. IL-33 has an extremely high level of mRNA expression in the brain.[18,21] The role of IL-33 involved in central nervous system inflammation indicated IL-33 may be a potential biomarker for multiple sclerosis.[21] In Chinese SLE patients, serum IL-33 levels are also upregulated significantly.[22] Importantly, serum IL-33 levels may be a promising indicator in RF + poly-JIA disease.[23] A study of the Chinese population was performed to estimate IL-33 polymorphisms on ankylosing spondylitis risk and there were statistically significant differences at SNPs rs1891385, rs1929992.[24] At the genetic polymorphism level, the rs1891385C allele of IL-33 had a significantly increased risk in Chinese SLE patients.[25]

As described above, these autoimmune diseases may share the same risk loci in genes of PADI4 and IL-33. And that they share some clinical characteristics may be the reason. However, none has ever been reported about the above SNPs in PADI4 and IL-33 of JSLE and JIA in a Chinese population. Thus, we will focus this study on associations between SNPs in PADI4 and IL-33 and the pathogenesis of JSLE and JIA in Southwest China.

2. Materials and methods

2.1. Subjects

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). The study group consisted of 607 subjects, including 303 healthy controls, 144 JSLE patients, and 160 JIA patients. All the subjects were recruited from West China Second University Hospital from November 2017 to August 2021. JSLE was diagnosed according to the revised American College of Rheumatology (ACR) criteria for the diagnosis of SLE before the age of 18 years.[26] JIA patients were diagnosed according to the International League of Associations for Rheumatology (ILAR) criteria.[27] 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 before 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 (ELISA) respectively (EUROIMMUN, Germany). Serum rheumatoid factor, Complement C3, and Complement C4 were measured by nephelometry (BN II, SIEMENS, Germany).

2.3. PADI4 and IL-33 polymorphisms

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

The information on SNPs in PADI4 and IL-33 genes were shown in Supplementary Table 1, http://links.lww.com/MD/H838. Primers for the 4 SNPs were shown in Supplementary Table 2, http://links.lww.com/MD/H839. The SNPs were genotyped using a Sequenom MassArray system (iPLEX assay, Sequenom) according to the manufacturer's 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 Figure 1, http://links.lww.com/MD/H840).

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 median and interquartile. Hardy–Weinberg equilibrium (HWE) 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 performed to explore whether PADI4 polymorphisms were in strong linkage disequilibrium or they independently contributed to the risk of JSLE, which also can capture additional significant variants since it's more sensitive than the single SNP analysis. Haplotypes with frequencies of >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).[28]

All the statistical analyses were performed using SPSS 21.0 software (IBM Corporation, New York) and Haploview software. Additional information on statistical methods is available in the Supplementary material. A two-sided *P* value of <.05 was deemed statistically significant.

3. Results

3.1. Demographic and clinical characteristics

The 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 1.00 years and 1.83 years. Among all the JSLE patients, the positive frequencies of ANA and LN were 90.97% and 44.44% respectively. 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 were 21.2%, 23.8%, and 20% respectively.

3.2. Association of PADI4 polymorphisms with the risk of JSLE and JIA

We genotyped 2 PADI4 SNPs (rs2240337 and rs2240340) in 303 healthy controls and 304 patients, including 144 JSLE patients and 160 JIA patients. The distributions of the

Table 1

Demographic and clinical characteristics of the study participants.

Variables	Healthy controls	JSLE	JIA
Number of subjects	303	144	160
Age, years, mean ± SD[a]	9.47 ± 3.68	12.24 ± 2.56	9.97 ± 3.58
Sex (male/female)	170/133	16/129	89/72
Disease duration (yr)[†]	-	1.83 (0.58–3.17)	1.00 (0.50–3.00)
Vitamin D (ng/mL)[†]	17.60 (12.98–24.80)	21.30 (15.65–28.05)	24.30 (18.58–28.58)
ANA (N [%])	-	131 (90.97%)	23 (14.38%)
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)[†]	-	9.00 (4.00–18.00)	13.00 (2.00–27.50)
Serum Complement C3 (g/L)[†]	-	0.74 (0.62–0.90)	1.08 (0.94–1.35)
Serum Complement C4 (g/L)[†]	-	0.14 (0.10–0.18)	0.22 (0.18–0.29)

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.

[a]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 peptidyl arginine deiminase type IV (PADI4) gene SNPs in patients with JSLE, JIA, and healthy controls.

					Controls vs JSLE		Controls vs JIA	
SNPs	Genotype/allele	Controls[a], *n* (%)	JSLE, *n* (%)	JIA[a], *n* (%)	Age- and gender-adjusted OR (95% CI)[†]	*P*[†]	Age- and gender-adjusted OR (95% CI)[†]	*P*[†]
rs2240337	TT	2 (0.6)	2(1.4)	1 (0.6)	1.00 (ref.)		1.00 (ref.)	
	CT	40 (13.4)	20 (13.9)	22 (13.8)	0.42 (0.05–3.84)	.44	1.20(0.10–14.54)	.89
	CC	257 (86.0)	122 (84.7)	137 (85.6)	0.41 (0.05–3.25)	.40	1.02(0.09–11.61)	.99
	CT + CC	297 (99.4)	142 (98.6)	159 (99.4)	0.40 (0.05–3.25)	.40	1.03(0.09–11.64)	.98
	CT + TT	42 (14.0)	22 (15.3)	23 (14.4)	1.00 (ref.)		1.00 (ref.)	
	CC	257 (86.0)	122 (84.7)	137 (85.6)	0.87 (0.45–1.70)	.69	0.98(0.57–1.70)	.94
	T	44 (7.4)	24 (8.3)	24 (7.5)	1.00 (ref.)		1.00 (ref.)	
	C	554 (92.6)	264 (91.7)	296 (92.5)	0.83 (0.45–1.52)	.54	0.98(0.59–1.65)	.95
	HWE	*P* = .95	*P* = .55	*P* = .99				
rs2240340	TT	59 (19.5)	14(9.7)	36 (22.6)	1.00 (ref.)		1.00 (ref.)	
	CT	137 (45.4)	86 (59.7)	71 (44.7)	**3.35 (1.62–6.94)**	**.001**	0.88(0.53–1.47)	.63
	CC	106 (35.1)	44 (30.6)	52 (32.7)	1.84 (0.86–3.96)	.12	0.80(0.47–1.36)	.41
	CT + CC	243 (80.5)	130 (90.3)	123 (77.4)	**2.63 (1.32–5.27)**	**.006**	0.85(0.53–1.36)	.50
	CT + TT	196 (64.9)	100 (69.4)	107 (67.3)	1.00 (ref.)		1.00 (ref.)	
	CC	106 (35.1)	44 (30.6)	52 (32.7)	0.74 (0.45–1.23)	.24	0.88(0.59–1.33)	.55
	T	255 (42.2)	114 (39.6)	143 (45.0)	1.00 (ref.)		1.00 (ref.)	
	C	349 (57.8)	174 (60.4)	175 (55.0)	1.13 (0.81–1.59)	.47	0.90(0.68–1.18)	.42
	HWE	*P* = .47	*P* = .01	*P* = .47				

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

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

[a]Missing data were excluded from the analyses for HC (rs2240337, n = 4; rs2240340, n = 1) and JIA (rs2240340, n = 1).

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

allele frequencies for the 2 SNPs complied with HWE both in the cases and controls among Chinese children ($P > .05$) (Table 2). As shown, the rs2240340 genotype was significantly associated with JSLE susceptibility {dominant model, OR = 2.63, 95% CI = 1.32–5.27, $P = .006$; additive model (CT vs TT): OR = 3.35, 95% CI = 1.62–6.94, $P = .001$} (Table 2). Nonetheless, similar allele and genotype frequencies were observed between the rs2240337 polymorphism and JSLE susceptibility ($P > .05$) (Table 2).

Moreover, no significant difference was observed between JIA patients and healthy controls in the allele frequency of PADI4 rs2240337 and PADI4 rs2240340 (rs2240337, OR = 0.98, 95% CI = 0.57–1.70, $P = .94$; rs2240340, OR = 0.90, 95% CI = 0.68–1.18, $P = .42$) (Table 2). Besides, there was no significant association between the genotype distributions and the JIA susceptibility ($P > .05$) (Table 2).

3.3. Association of IL-33 polymorphisms with the risk of JSLE and JIA

We also genotyped 2 SNPs IL-33 rs1891385 and IL-33 rs1929992 in 303 healthy controls, 144 JSLE patients and 160 JIA patients. The distributions of the allele frequencies for the 2 SNPs also complied with HWE both in the cases and controls in Chinese children ($P > .05$) (Table 3). No significant difference was observed between JSLE patients and healthy controls in the allele frequency of IL-33 rs1891385 and IL-33 rs1929992 (rs1891385, OR = 1.20, 95% CI = 0.82–1.74, $P = .34$; rs1929992, OR = 1.06, 95% CI = 0.75–1.48, $P = .75$) (Table 3). And also, there were similar genotype distributions for the two SNPs in the JSLE patients and healthy controls ($P > .05$) (Table 3).

Meanwhile, there was also no significant association between the 2 SNPs and the JIA susceptibility (rs1891385, OR = 0.98, 95% CI = 0.72–1.35, $P = .91$; rs1929992, OR = 1.24, 95% CI = 0.94–1.62, $P = .13$) (Table 3). Similar genotype distributions in IL-33 rs1891385 and IL-33 rs1929992 were also found in the JIA patients and healthy controls ($P > .05$) (Table 3).

3.4. Correlation of PADI4 and IL-33 polymorphisms with nephritis of JSLE patients

In the present study, the prognostic role that might be played by PADI4 rs2240337, PADI4 rs2240340, IL-33 rs1891385 and IL-33 rs1929992 in JSLE was examined (Table 4). Therefore, subgroup analysis was performed in JSLE patients with nephritis and without nephritis. Interestingly, we found there is a significant association between IL-33 rs1929992 polymorphism and reduced risk of nephritis in JSLE patients {recessive model (TT vs CC + CT): OR = 0.42, 95% CI = 0.18–0.96, $P = .041$} (Table 4). However, no significant genotype differences of PADI4 (rs2240337, rs2240340) and IL-33 rs1891385 polymorphisms were found between the 2 groups (recessive model: PADI4 rs2240337, OR = 0.47, 95% CI = 0.19–1.19, $P = .11$; PADI4 rs2240340, OR = 0.69, 95% CI = 0.33–1.42, $P = .31$; and IL-33 rs1891385, OR = 1.72, 95% CI = 0.56–5.27, $P = .35$) (Table 4).

3.5. Haplotype analysis of PADI4 with the risk of JSLE

Haplotypes were constructed upon the basis of 2 PADI4 SNPs (rs2240337 and rs2240340). Both PADI4 haplotype blocks in JSLE patients and healthy controls were constructed from rs2240337 (minor allele T) and rs2240340 (minor allele T). In the present study, no significant haplotype differences were observed between JSLE patients and healthy controls (block CC: Chi Square = 0.616, $P = .43$; block TC: Chi Square = 1.171, $P = .28$; block TT: Chi Square = 0.297, $P = .59$) (Table 5).

4. Discussion

PADI4 and IL-33 gene variants could affect the susceptibility to autoimmune diseases. However, little information was available about the role of IL-33 polymorphisms in the pathophysiology of JSLE and JIA. With AS patients, IL-33 SNPs (rs1891385, rs1929992) were detected at a significantly lower and higher level in the patients' group, respectively.[24] And our study revealed the relationship between rs1891385, rs1929992 polymorphism, and JSLE susceptibility together

Table 3

Genotype and allele analyses of the interleukin 33 (IL-33) gene SNPs in patients with JSLE, JIA, and healthy controls.

					Controls vs JSLE		Controls vs JIA	
SNPs	**Genotype/allele**	**Controls[a], *n* (%)**	**JSLE, *n* (%)**	**JIA, *n* (%)**	**Age- and gender-adjusted OR (95% CI)[†]**	***P*[†]**	**Age- and gender-adjusted OR (95% CI)[†]**	***P*[†]**
rs1891385	AA	167 (55.7)	72 (50.0)	91 (56.9)	1.00 (ref.)		1.00 (ref.)	
	AC	114 (38.0)	58 (40.3)	58 (36.2)	1.19 (0.72–1.97)	.50	0.94 (0.63–1.41)	.77
	CC	19 (6.3)	14 (9.7)	11 (6.9)	1.43 (0.59–3.44)	.43	1.05 (0.47–2.31)	.91
	AC + CC	133 (44.3)	72 (50.0)	69 (43.1)	1.23 (0.76–1.97)	.40	0.95 (0.65–1.41)	.81
	AC + AA	281 (93.7)	130 (90.3)	149 (93.1)	1.00 (ref.)		1.00 (ref.)	
	CC	19 (6.3)	14 (9.7)	11 (6.9)	1.34 (0.56–3.19)	.51	1.08 (0.50–2.34)	.84
	A	448 (74.7)	202 (70.1)	240 (75.0)	1.00 (ref.)		1.00 (ref.)	
	C	152 (25.3)	86 (29.9)	80 (25.0)	1.20 (0.82–1.74)	.34	0.98 (0.72–1.35)	.91
	HWE	$P = .99$	$P = .90$	$P = .91$				
rs1929992	CC	84 (28.1)	39 (27.1)	33 (20.6)	1.00 (ref.)		1.00 (ref.)	
	CT	145 (48.5)	70 (48.6)	85 (53.1)	0.98 (0.56–1.73)	.95	1.51 (0.93–2.46)	.10
	TT	70 (23.4)	35 (24.3)	42 (26.3)	1.09 (0.54–2.19)	.82	1.54 (0.88–2.71)	.13
	CT + TT	215 (71.9)	105 (72.9)	127 (79.4)	1.01 (0.59–1.73)	.96	1.54 (0.97–2.44)	.07
	CT + CC	229 (76.6)	109 (75.7)	118 (73.7)	1.00 (ref.)		1.00 (ref.)	
	TT	70 (23.4)	35 (24.3)	42 (26.3)	1.14 (0.65–2.00)	.64	1.16 (0.74–1.81)	.52
	C	313 (52.3)	148 (51.4)	151 (47.2)	1.00 (ref.)		1.00 (ref.)	
	T	285 (47.7)	140 (48.6)	169 (52.8)	1.06 (0.75–1.48)	.75	1.24 (0.94–1.62)	.13
	HWE	$P = .89$	$P = .95$	$P = .71$				

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

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

[a]Missing data were excluded from the analyses for HC (rs1891385, n = 3; and rs1929992, n = 4).

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

Table 4

Correlation of PADI4 and interleukin 33 (IL-33) polymorphisms with nephritis in juvenile systemic lupus erythematosus (JSLE) patients.

Genes	SNPs	Genotype	Without nephritis, *n* (%)	With nephritis, *n* (%)	Age- and gender-adjusted OR (95% CI)[a]	*P*[a]
PADI4	rs2240337	TT + CT	9 (11.2)	13 (20.3)	1.00 (ref.)	
		CC	71 (88.8)	51 (79.7)	0.47 (0.19–1.19)	.11
PADI4	rs2240340	TT + CT	53 (66.2)	47 (73.4)	1.00 (ref.)	
		CC	27 (33.8)	17 (26.6)	0.69 (0.33–1.42)	.31
IL-33	rs1891385	AA + CA	74 (92.5)	56 (87.5)	1.00 (ref.)	
		CC	6 (7.5)	8 (12.5)	1.72 (0.56–5.27)	.35
IL-33	rs1929992	CC + CT	55 (68.8)	54 (84.4)	1.00 (ref.)	
		TT	25 (31.2)	10 (15.6)	**0.42 (0.18–0.96)**	**.041**

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

CI = confidence interval, IL-33 = interleukin 33, PADI4 = peptidyl arginine deiminase type IV, OR = odds ratio.

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

Table 5

Two peptidyl arginine deiminase type IV (PADI4) gene SNPs haplotype frequencies identified in controls and patients with juvenile systemic lupus erythematosus (JSLE).

Haplotype	Freq.	Case, control ratio counts	Case, control frequencies	Chi square	*P* value
CC	0.585	173.8: 114.2, 347.7: 256.3	0.603, 0.576	0.616	.4326
TC	0.338	90.2: 197.8, 211.4: 392.6	0.313, 0.350	1.171	.2791
TT	0.076	23.8: 264.2, 43.6: 560.4	0.083, 0.072	0.297	.5858

with JIA. In our work, the PADI4 genotype (rs 2240340, CT, CT + CC) and IL-33 genotype (rs 1929992, TT) correlate with the risk of JSLE in the Southwest Chinese population significantly. Otherwise, no significant differences were discovered in these 2 genes polymorphisms analyses of JIA considering the age and gender. Often, different autoimmune diseases may share the same genetic variants. TT genotype of PADI4 rs2240340 was related to polyarticular JIA significantly in previous research, while the result was not in accordance with that of JIA patients in Southwest Chinese.[17] It may be the reason that subjects involved belonged to different populations with a diverse distribution of gender and age groups. Additionally, it may influence the activity of PADI4 and IL-33 for rs2240340 and rs1929992 polymorphisms, respectively. Still, no significant differences were illustrated when considering the age and gender of subjects for any PADI4 and IL-33 variants in JIA in this study. But PADI4 rs2240340 A allele was significantly associated with the risk of JIA in the Japanese populations.[16] All these data demonstrated that the correlation of PADI4 rs2240340 and IL-33 rs1929992 polymorphisms with JSLE was not the same as that in JIA patients. It suggested that the same related gene polymorphisms may have diverse and complicated distributions in different age stages or populations of patients with autoimmune diseases.

In summary, our article firstly revealed PADI4 polymorphisms (rs2240340, rs2240337) and IL-33 polymorphisms (rs1891385, rs1929992) with JSLE and JIA risk in the Southwest Chinese population. We found alleles of rs2240340 at gene PADI4 and rs1929992 at gene IL-33 were associated with JSLE risk in the Southwest Chinese population, whereas none was found for these polymorphisms analysis between JIA patients and healthy controls. Nevertheless, our results were obtained based on a limited population due to the long period for the sample collection. Importantly, we took age and sex into account when analyzing data, but the gene-environment interactions were left out of consideration. In addition, some other polymorphisms or splicing isoforms of PADI4 and IL-33 about JSLE and JIA pathogenicity should be concerned since more variants of them have been identified. Herein, more risk genes and polymorphisms would be focused on associations with JSLE and JIA risk. Furthermore, different kinds of juvenile autoimmune diseases may have the same SNPs of the gene, but the polymorphisms associations with the disease risk need more research.

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Author contributions

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