Genetic Association of rs15672 and rs12640056 Polymorphisms with the Susceptibility of Systemic Lupus Erythematosus in a Chinese Population

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Abstract

In this study, we aimed to investigate the relationship of IKBKE rs15672 and BANK1 rs12640056 gene polymorphisms with systemic lupus erythematosus (SLE) susceptibility in China. A case-control study was performed on 567 SLE patients and 345 healthy controls. Six single nucleotide polymorphisms (rs15672, rs2296164, rs12640056, rs6842661, rs1957106 and rs2274064) and clinical features were analyzed. Genotyping was executed with improved multiplex ligation detection reaction assay. SNP rs15672 increased the risk (P = 0.028, OR = 1.25, 95%CI = 1.02–1.52) but rs12640056 decreased the risk of SLE (P = 0.015, OR = 0.78, 95%CI = 0.64–0.95). For rs15672, patients carrying allele A assumed high-level IL-6 (25.36 ± 4.64 vs 16.56 ± 2.95, P = 0.036) and IL-4 (12.06 ± 4.51 vs 4.88 ± 1.76, P = 0.047), but low-level granzyme B (14.07 ± 1.86 vs 18.38 ± 3.85, P = 0.023), IL-18 (267.39 ± 14.67 vs 348.57 ± 44.25, P = 0.002) and IL-33 (0.91 ± 0.26 vs 2.19 ± 1.35, P = 0.029). Organ injury showed that mutant genotype at rs15672 had high-prevalence of arthritis (32.02%) and serositis (34.78%), but low in neuropathy (9.09%). For rs12640056, patients carrying allele T assumed low-level IL-33 (0.46 ± 0.17 vs 2.16 ± 1.00, P = 0.009). In conclusion, gene polymorphisms of rs15672 and rs12640056 present relevant with susceptibility of SLE, and affect the expression of cytokine and organ injury.

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic auto-inflammatory disorder that leads to multiple organ damage. Previous studies have pointed out multifactorial etiological factors including immunologic, environmental, genetic and other factors promote the occurrence of SLE.[1] Among them, genetic factors have been considered to contribute to the development and progression of SLE.[2] Recent studies on the human genome-wide association studies (GWAS) suggest that single nucleotide polymorphisms (SNPs) in protein-coding genes are significantly associated with SLE related organ damage.[3] Studies on twins and families also indicate that genetic susceptibility has a higher heritability and consistency rate in SLE families compared with sporadic populations.[4] The GWAS studies on the SLE families have been successfully applied to recognize more loci related to the susceptibility of SLE. Previous studies of our group have shown that gene polymorphisms of NF-κB (nuclear factor κB) signaling pathways were associated with SLE in a Chinese population.[5] Therefore, based on the above studies, we performed a case-control study on patient-specific SNPs related to NF-κB signaling pathway in sporadic cases of SLE and healthy controls (HCs). Then we aimed at investigating the association between patient-specific SNPs and susceptibility, disease activity, clinical subtypes, autoantibodies, and serum cytokines to further explore the potential mechanism of SLE.

2. Materials And Methods

2.1. Patients

A total of 919 samples were obtained from one SLE family (n = 7, including 3 confirmed SLE and 4 healthy members, the pedigree diagram drawn by Yongkang Wu), sporadic female SLE patients (n = 567) and healthy female controls (n = 345).[6] All the SLE family members in the study were diagnosed by the American College of Rheumatology (ACR) 1997 classification criteria. In this SLE family, the father died, the mother was not a SLE patient, but their four daughters were SLE patients with clinical manifestations. The elder sister died, other 3 SLE sisters (Specimen No. 2, 3 and 4) and their mother (Specimen No. 1) were selected as research specimens in this study. Children of the family haven't show SLE symptoms till now. The entire SLE family can be found as Supplementary Figure S1 online.

Sporadic SLE was diagnosed by rheumatologist according to the ACR 1997. Although the European League Against Rheumatism (EULAR)/ACR developed a new classification criteria in 2019, we still followed the classification criteria of ACR 1997 because of many years of data collection.[7] SLE disease activity in 567 patients was assessed by 2 experienced rheumatologists based on the SLEDAI-2K.[8] Patients with drug-induced SLE and without a complete medical history were excluded. Healthy female controls were selected from the health examination center of West China Hospital. None of these individuals had infectious diseases, autoimmune disorders or family history of autoimmune diseases.

This study was approved by the Ethics Committee of West China Hospital (Registration number: 20190559). All the experiments were performed according to the *Declaration of Helsinki's* ethical principles for medical research involving human subjects. Any forms of registration that may identify the patients were excluded from the content of the paper.

2.2. Clinical and laboratory data

Demographic and clinical data were collected from the medical records. Laboratory tests of immunocorrelation included the following items: immunoglobin G (IgG), IgA, IgM, complement 3 (C3) and C4. Serum samples were tested for antinuclear antibody (ANA) by immunofluorescent (IIF) assay coated with HEp-2 cells (Euroimmun, Germany). Anti-dsDNA, anti-Sm, anti-ribonuclear protein (anti-RNP), anti-SSA/Ro52, anti-SSB/Ro60 and anti-RIB antibodies were detected by line immunoassays (Euroimmun, Germany). The serum levels of cytokines including interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-17A, IL-18, IL-33, IL-21, IP-10, granzyme B, interferonγ(IFNγ) and tumor necrosis factorα(TNFα) were detected in SLE patients (R&D, America).

2.3. Specific SNPs genotyping and linkage disequilibrium evaluation

We selected patient-specific SNPs based on the results of previous genealogical study at first.[6] Patient-specific SNPs were defined as SNPs that presented in all three patients but not in their mother. Healthy-specific SNPs were defined as SNPs that presented only in the mother. Finally, gene polymorphism of rs15672, rs2296164, rs12640056, rs6842661, rs1957106 and rs2274064 were analyzed in sporadic SLE patients and HCs by improved multiplex ligation detection reaction (iMLDR) (Genesky Biotechnologies Inc, China). Some of the samples were randomly selected for direct sequencing to confirm the results genotyped using iMLDR. The haploview software (version 4.2) was used to perform linkage disequilibrium (LD) evaluation for combination of SNPs by calculating the r^2 coefficient in pairs.

2.4. Statistical analysis

The statistical analyses were applied by using plink (version 1.9) and statistical package for the social sciences (SPSS) software (version 22.0). Hardy–Weinberg equilibrium (HWE) was performed for the polymorphisms by 2-sided chi-square test. Differences in allele genotypic model were evaluated with chi-square test. Allele model included dominant model, recessive model, co-dominant model. The association between genetic polymorphisms and cytokines/laboratory data were evaluated by one-way analysis of variance (ANOVA) and followed by Tukey multiple comparisons (equal variances) or Dunnett's T3 test (unequal variances). Odds ratios (ORs) and 95% confidence interval (*CI*) were calculated by logistic regression model between different groups. A P value < 0.05 was considered statistically significant.

3. Results

3.1. The main characteristics of the study population

We used haploview (version 4.2) to construct LD analysis of rs15672, rs2296164, rs12640056, rs6842661, rs1957106 and rs2274064. Samples were collected from sporadic SLE cases (n = 567) and HCs (n = 345) in this study. The result showed that there were strong LD between rs12640056 and rs6842661 ($r^2 = 1$), rs2296164 and rs2274064 ($r^2 = 0.95$), as illustrated in Fig. 1.

3.2. Polymorphisms of patient-specific SNPs association with SLE susceptibility

A total of 912 participants, including 567 SLE patients and 345 HCs, were involved in this gene polymorphisms of rs15672, rs2296164, rs12640056, rs6842661, rs1957106 and rs2274064. However, only rs15672 and rs12640056 were associated with SLE susceptibility, as shown in Table 1. The negative results can be found as Supplementary Table S1 online. Cohorts showed no significant deviation from HWE for genotyped SNPs (all $P > 0.05$). When comparing allele frequencies of rs15672 and rs12640056 between SLE patients and HCs, significant differences were indicated. SNP rs15672 increased the risk of SLE ($P = 0.028$, OR = 1.25, 95%CI = 1.02–1.52). and rs12640056 decreased the risk of SLE ($P = 0.015$, OR = 0.78, 95%CI = 0.64–0.95). With further validation, we found that AA + GA genotype at rs15672 (Dominant model: $P = 0.033$, OR = 1.35, 95%CI = 1.03–1.78) and TT + CT genotype at rs12640056 (Dominant model: $P = 0.011$, OR = 0.71, 95%CI = 0.54–0.92; Co-dominant model: $P = 0.020$, OR = 0.71, 95%CI = 0.54–0.95) were associated with SLE susceptibility.

Table 1
Genotype and allele frequencies of patient-specific SNPs in SLE patients and controls.

SNP	Model	Genotype/ allele	SLE, N (%)	Controls, N (%)	OR (95% CI)	*P*
rs15672	Dominant	AA + GA	364 (64.20)	197 (57.10)	1.35 (1.03–1.78)	**0.033**
		GG	203 (35.80)	148 (42.90)	1.00 (ref)	-
	Recessive	AA	94 (16.58)	46 (13.34)	1.29 (0.88–1.89)	0.187
		GA + GG	473 (83.42)	299 (86.66)	1.00 (ref)	-
	Co-dominant	AA	94 (16.58)	46 (13.34)	1.49 (0.99–2.25)	0.057
		GA	270 (47.62)	151 (43.77)	1.30 (0.98–1.74)	0.074
		GG	203 (35.80)	148 (42.89)	1.00 (ref)	-
	Allele	A	458 (40.39)	243 (35.22)	1.25 (1.02–1.52)	**0.028**
		G	676 (59.61)	447 (64.78)	1.00 (ref)	-
rs12640056	Dominant	TT + CT	273 (48.15)	196 (56.81)	0.71 (0.54–0.92)	**0.011**
		CC	294 (51.85)	149 (43.19)	1.00 (ref)	-
	Recessive	TT	55 (9.70)	41 (11.88)	0.80 (0.52–1.22)	0.297
		CT + CC	512 (90.30)	304 (88.12)	1.00 (ref)	-
	Co-dominant	TT	55 (9.70)	41 (11.88)	0.68 (0.43–1.07)	0.092
		CT	218 (38.45)	155 (44.93)	0.71 (0.54–0.95)	**0.020**
		CC	294 (51.85)	149 (43.19)	1.00 (ref)	-
	Allele	T	328 (28.92)	237 (34.35)	0.78 (0.64–0.95)	**0.015**
		C	806 (71.08)	453 (65.65)	1.00 (ref)	-

SNPs = single nucleotide polymorphisms, SLE = systemic lupus erythematosus.

3.3. Gene polymorphisms association with clinical data between SLE patients.

SLE patients often had variations in disease severity and organ injury. In this study, we attempted to examine the effects of gene polymorphism on clinical parameters and phenotypes in SLE patients. A total of 400 SLE patients with complete medical records participated in this part. The positive results and

related negative results were summarized in Table 2. The results showed that rs15672 and rs12640056 gene polymorphisms were associated with change of IgM and extractable nucler antigen (ENA) antibodies in dominant model between SLE patients. SLE cases with mutant genotype at rs15672 had higher IgM level (1158.09 vs 1005.39, *P* = 0.014) and prevalence of anti-SSB (14.62% vs 7.48%, *P* = 0.034), but lower C3 level (0.51 vs 0.52, *P* = 0.036) and prevalence of anti-RIB (28.46% vs 40.14%, *P* = 0.016). While mutant genotype at rs12640056 had higher IgM level (1168.57 vs 1039.09, *P* = 0.001), but lower prevalence of anti-dsDNA (60.00% vs 70.00%, *P* = 0.036). The other negative results were illustrated in Table 2.

Table 2
Association between gene polymorphisms with clinical data in SLE patients.

Clinical characteristics	rs15672 Median or genotype frequency		*χ2*	*P*	rs12640056 Median or genotype frequency		*χ2*	*P*
	Mutant (n = 253)	Wild (n = 147)			Mutant (n = 200)	Wild (n = 200)		
Immunoglobin G (g/L)	15.22 (7.85–22.58)	13.85 (6.95–20.74)	0.717	0.398	14.92 (7.65–22.18)	14.53 (7.34–21.72)	0.109	0.741
Immunoglobin A (g/L)	2683.90 (1404.58-3963.22)	2671.90 (1273.82-4069.98)	0.129	0.720	2704.67 (1334.05-4075.28)	2655.61 (1378.97-3932.26)	0.036	0.849
Immunoglobin M (g/L)	1158.09 (370.59-1945.59)	1005.39 (369.19-1641.59)	6.139	**0.014**	1168.57 (325.47-2011.67)	1039.09 (420.79-1657.39)	10.498	**0.001**
Complement 3 (g/L)	0.51 (0.27–0.75)	0.52 (0.31–0.72)	4.451	**0.036**	0.50 (0.27–0.73)	0.53 (0.30–0.75)	0.022	0.882
Complement 4 (g/L)	0.11 (0.04–0.17)	0.11 (0.05–0.16)	2.849	0.092	0.10 (0.04–0.17)	0.11 (0.05–0.17)	0.023	0.880
Anti-dsDNA, n (%)	163 (64.43)	97 (65.98)	0.099	0.753	120 (60.00)	140 (70.00)	4.396	**0.036**
Anti-RNP, n (%)	136 (53.75)	81 (55.10)	0.068	0.794	106 (53.00)	111 (55.50)	0.252	0.616
Anti-sm, n (%)	72 (28.46)	46 (31.29)	0.359	0.549	58 (29.00)	60 (30.00)	0.048	0.826
Anti-SSA, n (%)	147 (58.10)	83 (56.46)	0.102	0.749	116 (58.00)	114 (57.00)	0.041	0.840
Anti-SSB, n (%)	37 (14.62)	11 (7.48)	0.490	**0.034**	20 (10.00)	28 (14.00)	1.515	0.218
Anti-RIB, n (%)	72 (28.46)	59 (40.14)	5.757	**0.016**	70 (35.00)	61 (30.50)	0.919	0.338

SLE = systemic lupus erythematosus.

3.4. Gene polymorphisms association with organ injury between SLE patients.

Previous results (Table 1) had showed that rs15672 and rs12640056 gene polymorphisms were associated with SLE susceptibilities. Therefore, we further analyzed the relationship between different gene subtypes and organ injury in the dominant model. We also included 400 SLE patients with complete medical records in this part. Analysis performed on dominant model indicted that patients with rs15672 A allele had significantly higher prevalence of arthritis (32.02% vs 20.41%, *P* = 0.012) and serositis (34.78% vs 25.17%, *P* = 0.046) than GG genotype, while patients with rs15672 A allele had significantly lower prevalence of neuropathy (9.09% vs 15.65%, *P* = 0.048). And patients with rs12640056 T allele showed no significant differences in organ injury (Table 3).

Table 3
Association between polymorphisms of rs15672 and rs12640056 with organ injury on dominant model.

Characteristics	SLE with/without organ injury (rs15672)				SLE with/without organ injury (rs12640056)			
	Wild (n = 147)/Mutant (n = 253)	χ2	P	OR (95%CI)	Wild (n = 200)/Mutant (n = 200)	χ2	P	OR (95%CI)
Lupus nephritis, n (%)	89 (60.54)/165 (65.22)	0.876	0.349	1.222 (0.803–1.860)	125 (62.50)/129 (64.50)	0.173	0.678	1.090 (0.725–1.638)
Neuropathy, n (%)	23 (15.65)/23 (9.09)	3.926	**0.048**	0.539 (0.291-1.000)	22 (11.00)/24 (12.00)	0.098	0.754	1.103 (0.597–2.040)
Hematologic disorders, n (%)	44 (29.93)/86 (33.99)	0.699	0.403	1.205 (0.778–1.869)	63 (31.50)/67 (33.50)	0.182	0.669	1.095 (0.721–1.665)
Arthritis, n (%)	30 (20.41)/81 (32.02)	6.248	**0.012**	1.837 (1.136–2.969)	55 (27.50)/56 (28.00)	0.012	0.911	0.975 (0.630–1.511)
Skin lesions, n (%)	57 (38.78)/102 (40.32)	0.092	0.761	1.067 (0.703–1.617)	74 (37.00)/85 (42.50)	1.263	0.261	1.259 (0.843–1.880)
Serositis, n (%)	37 (25.17)/88 (34.78)	3.999	**0.046**	1.586 (1.007–2.495)	64 (32.00)/61 (30.50)	0.105	0.746	1.072 (0.702–1.637)
Pericarditis, n (%)	43 (29.25)/82 (32.41)	0.432	0.511	1.160 (0.745–1.805)	60 (30.00)/65 (32.50)	0.291	0.590	1.123 (0.736–1.715)
SLE = systemic lupus erythematosus.								

Further gene polymorphisms at rs15672 were performed in neuropathy, arthritis and serositis. The results showed that the incidence of arthritis in rs15672 A allele were significantly higher than T allele (χ2 = 6.249, *P* = 0.044), while the difference between GA and AA types was not statistically significant (Table 4).

Table 4
Incidence of arthritis in different genotypes of rs15672 in SLE patients.

Characteristics	SLE with/without organ injury (rs15672)			*χ2*	*P*
	Genotype AA (n = 72)	Genotype GA (n = 181)	Genotype GG (n = 147)		
Neuropathy, n (%)	6 (8.34%)	17 (9.39%)	23 (15.65%)	3.983	0.137
Arthritis, n (%)	23 (31.94%)	58 (32.04%)	30 (20.41%)	6.249	0.044
Serositis, n (%)	28 (38.89)	60 (33.15%)	37 (25.17%)	4.789	0.091
SLE = systemic lupus erythematosus.					

3.5. Comparison between rs15672 and rs12640056 with cytokines in dominant model between SLE patients.

We attempted to examine the effects of dominant model with cytokines in SLE patients. We randomly chose 202 SLE patients for quantification of IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-18, IL-33, IL-21, IP-10, granzyme B, IFNγand TNFαcytokines using the R&D Human Inflammation Assay (Bio-Techne, Minneapolis, MN, USA). The results were summarized in Table 5.

Table 5
Comparison between cytokines in dominant model of rs15672 and rs12640056 in SLE patients.

SNP	Genotype	IL-6,mean ± SD (pg/ml)	*χ2*	*P*	Granzyme B,mean ± SD (pg/ml)	*χ2*	*P*	IL-4,mean ± SD (pg/ml)	*χ2*	*P*	IL-18,mean ± SD (pg/ml)	*χ2*	*P*	IL-33, mean ± SD (pg/n
rs15672	AA + GA (n = 124)	25.36 ± 4.64	4.468	**0.036**	14.07 ± 1.86	5.274	**0.023**	12.06 ± 4.51	4.027	**0.047**	267.39 ± 14.67	10.334	**0.002**	0.91 ± 0.26
	GG (n = 78)	16.56 ± 2.95			18.38 ± 3.85			4.88 ± 1.76			348.57 ± 44.25			2.19 ± 1.35
rs12640056	TT + CT (n = 89)	21.09 ± 4.31	0.411	0.522	14.96 ± 2.72	0.187	0.666	10.36 ± 4.69	0.260	0.611	301.41 ± 21.95	0.702	0.403	0.46 ± 0.17
	CC (n = 113)	22.57 ± 4.34			16.47 ± 2.67			7.93 ± 3.22			299.25 ± 31.52			2.16 ± 1.00

3.5. Comparison between rs15672 and rs12640056 with cytokines in dominant model between SLE patients.

We attempted to examine the effects of dominant model with cytokines in SLE patients. We randomly chose 202 SLE patients for quantification of IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-18, IL-33, IL-21, IP-10, granzyme B, IFNγand TNFαcytokines using the R&D Human Inflammation Assay (Bio-Techne, Minneapolis, MN, USA). The results were summarized in Table 5. The other negative results can be found as Supplementary Figure S2 online.

The analysis performed on dominant model indicted that rs15672 and rs12640056 were also associated with serum levels of IL-6, granzyme B, IL-4, IL-18 and IL-33. The dominant model results showed that AA + GA genotypes at rs15672 had higher IL-6 (25.36 ± 4.64 vs 16.56 ± 2.95, $P = 0.036$) and IL-4 (12.06 ± 4.51 vs 4.88 ± 1.76, $P = 0.047$), but lower granzyme B (14.07 ± 1.86 vs 18.38 ± 3.85, $P = 0.023$), IL-18 (267.39 ± 14.67 vs 348.57 ± 44.25, $P = 0.002$) and IL-33 (0.91 ± 0.26 vs 2.19 ± 1.35, $P = 0.029$) than GG genotype. However, the dominant model showed that only IL-33 (0.91 ± 0.26 vs 2.19 ± 1.35, $P = 0.009$) was significantly reduced in TT + CT genotypes at rs12640056. Other cytokines in the dominant model at rs12640056 showed no significant difference ($P > 0.005$).

4. Discussion

SLE is not an independent disease, but a complex disease with heterogeneous sub-phenotypes. Currently, a large number of evidence suggests that the pathogenesis of SLE has a strong genetic basis. Studies on SNPs polymorphisms in SLE have shown that patients with different races and regions may have different types of genetic mutations. However, studies of multiple members in the same family have shown that patient-specific SNPs in families may be a major causative factor for SLE.[9] Therefore, based on the genetic screening of SLE family, we selected further verification in the sporadic cases of SLE patients. Compared with previous reports, this study focused on the association of rs15672 and rs12640056 gene polymorphisms involved in NF-κB signaling pathway with SLE susceptibility and clinical characteristics. Some characteristics of family specific SNPs in the Southwest Chinese SLE patients were notable.

IKBKE gene is localized on chromosomal locus 1q32 and encodes inhibitor of κB kinase ε (IKKε) light chain, which participates in NF-κB signaling pathway.[10] Inflammatory cytokines like TNF-α, IL-1, IL-6 and IFN-γ stimulated the up-regulated expression of IKBKE.[11] IKBKE was phosphorylated under the regulation of the kinase complex. In addition, the lack of IKKε actived tumor necrosis factor (TNF) which was relate to activation NF-κB signaling pathway. As a result, IKBKE/TBK1 (TRAF family member-associated NF-κB activator-binding kinase 1, TBK1) mediated Lys63-linked polyubiquitination and activated the NF-κB pathway.[12] Previous studies have indicated that IKBKE rs1539241, rs12142086 and rs2151222 were associated with NF-κB pathway.[10] GWAS study showed that IKBKE rs2297550 polymorphism was correlated with SLE.[13]

BANK1 gene is localized on chromosomal locus 4q24 and encodes a B-cell specific scaffold protein. BANK1 binds to BLK gene, promoting inositol 1, 4, 5-trisphosphate and its receptors and activates downstream genes. Polymorphism of BANK1 has been shown to be associated with susceptibility to systemic lupus erythematosus.[14] Recent studies have shown that Bank1 and NF-κB subunit 1 played critical regulatory role in antinucleolar antibodies in mercury-induced autoimmunity in mice.[15] However, the association of IKBKE and BANK1 gene polymorphisms of SLE in the Han population of southwest China remained unclear. Based on this background, this study investigated the association of IKBKE rs15672 and BANK1 rs12640056 gene polymorphisms and susceptibilities of SLE in Han population of southwest China.

Our results from Table 2 showed that A allele at the IKBKE rs15672 locus was 1.25 times the risk of developing SLE in the population carrying the G allele and T allele at the BANK1 rs12640056 locus was 0.78 times the risk in the population carrying the C allele. The results suggested that rs15672 A allele was a candidate risk allele while rs12640056 T allele was a protective allele for SLE in the Chinese Han population. It was worth noting that heterozygous mutation at rs15672 and rs12640056 accounted for higher proportions in SLE compared with homozygous mutation. rs15672 and rs12640056 were located in the 3' untranslated region (UTR), and the mutation of this site could further regulate the expression of gene by regulating the transcriptional function of mRNA.[16] The above data supported the significance of regional SNP gene polymorphisms related to the transcriptional regulatory function of gene.

We also attempted to explore the relationship between serum cytokines and organ injury in the dominant model. The results showed that serum levels of IL-6, granzyme B, IL-4, IL-18 and IL-33 in rs15672 A allele were significantly different from that of GG genotype. Compared with CC genotype, rs12640056 T allele only showed a decrease in IL-33. Recent study showed that CD95 and fas-associated via death domain (FADD) could enhance lipopolysaccharide (LPS)-induced NF-κB responses by activating macrophages and cause increased production of IL-6.[17] Another study showed that IL-6 enhanced the activity of the promoter of miR-34a by activating the NF-κB/p65 signaling pathway, and further attenuated the expression of forkhead box P3 (Foxp3) by targeting the 3' UTR.[18] Granzyme B was derived from cytotoxic lymphocytes and cytoplasmic granules released by natural killer cells. It mediated apoptosis by activating caspase. CD137 agonist enhanced the secretion of granzyme B by $CD8^+$T cells, which was activated by NF-κB nuclear translocation.[19] One study showed that SLE patients had a significant decreased percentage of granzyme B in B cells with disease activity and lupus nephritis.[20] IL-4 was a cytokine produced by activated T lymphocytes. It was involved in B cell proliferation and differentiation and stimulated the production of immunoglobulin IgE by B cells. Bach2-/- mice model showed that transcription factor Bach2-deficient follicular helper T (Tfh) cells were skewed toward the IL-4 producing subset, which induced IgG1 and IgE transformation of B cells.[21]

As an inflammatory cytokine, IL-18 can induce the synthesis of interferonγ(IFNγ). Studies supported the role of IL-18 in promoting organ injury of SLE. IL-18 mRNA was positively correlated with SLEDAI and anti-dsDNA antibody.[22] Studies on IL-18 gene polymorphisms and SLE organ injury showed that IL-18 (-1297C) were associated with renal involvement, and CC haplotype was associated with serositis.[23] Our study showed that IL-18 of rs15672 A allele was significantly higher than that of GG genotype. The correlation between IKBKE gene and IL-18 needs further confirmation. Recent studies on IL-33 in SLE have shown significant differences between IL-33, C-reactive protein (CRP) and erythrocyte regulatory rate (ESR).[24] Studies have shown that haplotype

rs1929992G and rs7044343T were both risk factors for SLE.[25] In this study, we found that IL-33 level was significantly lower in TT genotype of rs12640056. These results suggested that the relationship between rs12640056 polymorphisms and IL-33 may also be significant in the pathogenesis of SLE.

We also found that the carriers of rs15672 A allele might be involved in the development of clinical laboratory characteristics and organ injury in SLE. Compared with the wild group, the proportion of arthritis and serositis was increased, while the proportion of neuropathy was significantly decreased. Laboratory results showed the increased proportion of IgM and anti-SSB and the decreased proportion of C3 and anti-RIB. Studies have shown that C3 in active SLE patients was significantly lower than that in inactive SLE patients. The ratio of complement fission products iC3b and serum C3 in patients with SLE was correlated with SLE activity.[26] Another study showed that the activation product of C3, such as iC3b/C3dg in patients with SLE was significantly higher than that in healthy individuals.[27] This study showed that rs15672 A allele mutation was a risk factor for SLE and was associated with the decline level of C3 in SLE patients. A variety of autoantibodies and inflammatory factors in the blood of NPSLE patients can cause damage to the blood-brain barrier. Anti-ribosomal P protein (anti-RIB) antibody could bind to neuronal surface protein, causing calcium flow and neuronal apoptosis, further leading to nervous system damage.[28] The meta-analysis supported the possibility of anti-RIB in the pathogenic role of neuropsychiatric SLE (NPSLE).[29] We found that the percentage of anti-RIB and proportion of neuropathy in rs15672 A allele were decreased in this study.

Anti-SSB antibody is one of the common autoantibodies in the serum of SLE patients. A retrospective study showed a positive rate of secondary Sjögren syndrome (SLE-sSS) in adult SLE was 23%.[30] Further evaluation showed that anti-SSB antibody was associated with alopecia. In anti-SSB positive SLE patients, the incidence of alopecia, serositis and secondary Sjögren's syndrome (sSS) was higher than the negative group.[31] We found that the percentage of anti-SSB and proportion of serositis in rs15672 A allele were increased in this study. It was also worth noting that the incidence of sSS was relatively high. At the same time, systemic inflammatory state with higher levels of proinflammatory cytokines was found in SLE-sSS subgroup.[30] Therefore, the relationship between gene polymorphisms and SLE organ injury should be subdivided into SLE subgroups on the basis of expanding the sample size.

IgM autoantibodies appeared earlier than IgG in humoral immunity and regulated immune function in cardiovascular disease by identifying lipoproteins exposed during apoptosis and oxidative epitopes on phospholipid cell membranes.[32] IgM-antibodies against phosphorylcholine (anti-PC) was significantly reduced in SLE patients with cardiovascular disease (CVD) and atherosclerosis risk.[33] One study showed that IgM anti-cardiolipin and IgM anti-dsDNA were significantly higher in patients without renal disease. IgM anti-PC was also higher in patients with low disease activity.[34] In this study, the IgM of rs15672 A allele and rs12640056 T allele were higher than the wild groups. However, there was no difference in lupus nephritis and pericarditis between different groups. Previous study had shown that the rate of rheumatoid factor (RF) in SLE patients was high.[35] In this study, the incidence of arthritis was significantly increased in the rs15672 A allele, which suggested that IgM might be correlated with arthritis in SLE patients. We also noted that the diagnostic criteria for arthritis were derived from the medical records in this study. We should collect more relevant imaging data to describe the pericarditis more specifically.

In summary, we identified two loci, rs15672 and rs12640056, associated with SLE risk in Chinese Han population. IKBKE rs15672 gene polymorphism was related to SLE susceptibility and cytokine. rs15672 A allele increased the risk of SLE. It could increase the risk of arthritis and serositis, but reduce the risk of neuropathy. It was also associated with the high expression of serum cytokines IL-4 and IL-6. BANK1 rs12640056 polymorphism was also associated with SLE susceptibility and cytokine, but not with the organ injury of SLE. rs12640056 T allele could reduce the risk of SLE and was associated with low expression of serum cytokine IL-33.

Abbreviations

ACR = American College of Rheumatology

ANA = antinuclear antibody

ANOVA = analysis of variance

anti-PC = antibodies against phosphorylcholine

CI = confidence interval

CRP = C-reactive protein

CVD = cardiovascular disease

C3 = complement 3

ENA = extractable nuclear antigen

ESR = erythrocyte regulatory rate

EULAR = European League Against Rheumatism

FADD = fas-associated via death domain

Foxp3 = forkhead box P3

GWAS = genome-wide association studies

HCs = healthy controls

HWE = Hardy-Weinberg equilibrium

IFNγ= interferonγ

Ig = immunoglobin

IIF = immunofluorescent

IKKε = inhibitor of κB kinase ε

IL = interleukin

LPS = lipopolysaccharide

iMLDR = improved multiplex ligation detection reaction

LD = linkage disequilibrium

NF-κB = nuclear factorκB

NPSLE = neuropsychiatric SLE

OR = odds ratio

RF = rheumatoid factor

RIB = ribosomal P protein

RNP = ribonuclear protein

SLE = systemic lupus erythematosus

SLEDAI-2K = systemic lupus erythematosus disease activity index-2000

SNPs = single nucleotide polymorphisms

SPSS = statistical package for the social sciences

sSS = secondary Sjögren's syndrome

TBK1 = TRAF family member-associated NF-κB activator-binding kinase 1

Tfh = follicular helper T

TNFα= tumor necrosis factorα

TRAF = TNF receptor associated factor

UTR = untranslated region

WES = whole-exome sequencing

Declarations

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Author contributions

N.D.Z., J.Z.Z. and P.Z. collected and analyzed the data. N.D.Z. and Y.K.W. conceived and designed the experiments. N.D.Z., J.Z.Z., Y.B.L and Y.K.W. performed the experiments. J.L.Z., B.C. and Y.K.W. contributed reagents, materials, analysis tools. N.D.Z. wrote the manuscript. Y.K.W. was responsible for important academic content, including experimental design and implementation, manuscript drafting and critical revision. All authors approved the final version of the article and the authorship list.

Competing interests

The authors declare no competing interests.

Additional information

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Compliance with ethical standards

The study protocol and data analyses were approved by Ethics Committee of West China Hospital (Registration number: 20190559).

Statement of informed consent

All participants gave informed consent for this study.

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Figures

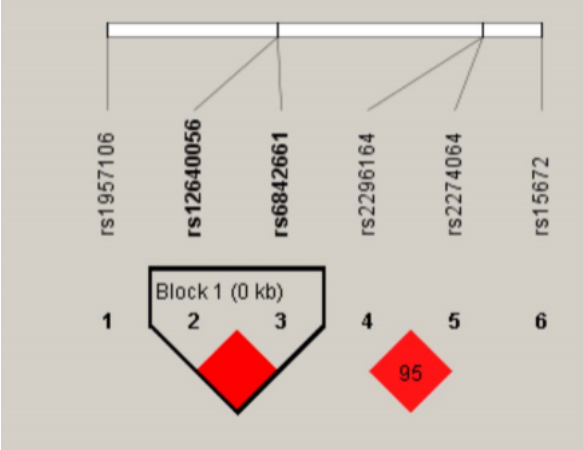

Figure 1

Linkage disequilibrium (LD) for six SNPs in 912 individuals. The LD plot shows the D'value between each pair of SNPs. The LD plot shows pairwise correlation coefficients (r2). Strong LD was represented by a higher percentage and a red square. (Haploview 4.2 cited from https://sourceforge.net/projects/haploview/). SLE = systemic lupus erythematosus, LD = linkage disequilibrium.

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