

Recapitulation of Candidate Systemic Lupus Erythematosus-Associated Variants in Koreans

Ki-Sung Kwon^{1,2}, Hye-Young Cho^{1,2}, Yeun-Jun Chung^{1,2*}

¹Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul 06591, Korea, ²Integrated Research Center for Genome Polymorphism, College of Medicine, The Catholic University of Korea, Seoul 06591, Korea

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple organ systems. Although the etiology of SLE remains unclear, it is widely accepted that genetic factors could be involved in its pathogenesis. A number of genome-wide association studies (GWASs) have identified novel single-nucleotide polymorphisms (SNPs) associated with the risk of SLE in diverse populations. However, not all the SNP candidates identified from non-Asian populations have been validated in Koreans. In this study, we aimed to replicate the SNPs that were recently discovered in the GWAS; these SNPs have not been validated in Koreans or have only been replicated in Koreans with an insufficient sample size to conclude any association. For this, we selected five SNPs (rs1801274 in *FCGR2A* and rs2286672 in *PLD2*, rs887369 in *CXorf21*, rs9782955 in *LYST*, and rs3794060 in *NADSYN1*). Through the replication study with 656 cases and 622 controls, rs1801274 in *FCGR2A* was found to be significantly associated with SLE in Koreans (odds ratio, 1.26, 95% confidence interval, 1.06 to 1.50; $p = 0.01$ in allelic model). This association was also significant in two other models (dominant and recessive). The other four SNPs did not show a significant association. Our data support that *FCGR* polymorphisms play important roles in the susceptibility to SLE in diverse populations, including Koreans.

Keywords: FCGR2A, genomewide association studies, single-nucleotide polymorphism, systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a chronic heterogeneous systemic autoimmune disease that can, in principle, affect any kind of body organ or cell, primarily in young women [1]. In SLE, altered immune responses may stimulate the production of autoantibodies and immune complex deposits in body organs, resulting in organ damage [2]. Like other autoimmune diseases, SLE has a complex pathogenesis, and the disease mechanism is still unclear; however, it is widely accepted that genetic factors could be important contributors to SLE [2, 3]. Accordingly, several lines of evidence have supported that SLE may be explained by genetic factors. For instance, the concordance rate of SLE in monozygotic twins (24% to 58%) is higher than the rate in dizygotic twins (2% to 5%), and also, the disease heritability is high (>66%) [4-6].

A number of genome-wide association studies (GWASs)

have identified novel single-nucleotide polymorphisms (SNPs) associated with the risk of SLE, such as *HLA*, *PTPN22*, *STAT4*, *IRF5*, *TNFAIP3*, *BLK*, and *BANK1*, in various ethnic groups, but most of the GWASs have been performed in Caucasians [1, 7-13]. In Korea, most of the previously reported SNPs were validated by GWASs, and potentially East Asian-specific SNPs, such as the SNPs in *ETS1*, *IKZF1*, and *SLC15A4*, were also identified [14-16]. Recently, Bentham *et al.* [12] performed a large-scale GWAS with 7,219 cases and 15,991 controls in Europeans and defined 43 SLE loci, including 10 new loci.

Among the SLE-associated loci in Bentham *et al.* [12], some of them have not been replicated in the Korean population, and others were replicated but not concluded to be associated due to a limited sample size. In this study, we aimed to replicate five SNPs that were not replicated or concluded to be association in Koreans.

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*Corresponding author: Tel: +82-2-2258-7343, Fax: +82-2-537-0572, E-mail: yejun@catholic.ac.kr

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Methods

Study subjects

All participants were of Korean ancestry. For the replication study, 656 SLE patients (males, 11; females, 645; age [mean \pm SD], 33.7 \pm 12.1 years) were recruited from the Rheumatology Center at Seoul St. Mary's Hospital, The Catholic University of Korea, South Korea. All patients had been diagnosed as having SLE according to the American College of Rheumatology-revised criteria for SLE [17, 18]. In addition, the allele frequencies for healthy Korean population as control were derived from the Korea Reference Genome Database (n=622). This study was performed under the approval of our institutional review board (MC12TIMI0026).

SNP genotyping

In this study, we selected five SNPs (rs1801274 in *FCGR2A* locus, rs2286672 in *PLD2* locus, rs887369 in *CXorf21* locus, rs9782955 in *LYST*, and rs3794060 in *NADSYN1*) that were identified to be associated with SLE from a previous GWAS [12] but have not been tested in a Korean population. Five SNPs were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) for allelic discrimination. TaqMan polymerase chain reaction (PCR) and fluorescence measurements were performed using a ViiA 7 Real-Time PCR System (Applied Biosystems) using a 384-well plate according to the manufacturer's instructions.

Statistical analysis

Chi-square test was used to estimate Hardy-Weinberg equilibrium with the genotype data of the SLE cases and controls. Significant differences in alleles and genotype frequencies between cases and controls were assessed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Odds ratios (ORs) were calculated by case and control allele frequencies with 95% confidence intervals (CIs). The p-values < 0.05 were regarded as statistically significant.

Results

Candidate SNP selection

Of the 43 SLE-associated loci identified by Bentham *et al.* [12], we narrowed down the candidate SNPs for this study per the following criteria: SNPs that had not been replicated in the Korean population, SNPs with a minor allele frequency above 5% in an East Asian population, and SNPs that were replicated in the Korean population but inconclusive owing to the limited sample size. Seven of the 43 SNPs were not replicated in the Korean population, and five of them showed a minor allele frequency above 5% in the East Asian population (Supplementary Table 1). Therefore, we selected the five SNPs for a replication study in Koreans (rs1801274 in *FCGR2A* locus, rs2286672 in *PLD2* locus, rs887369 in *CXorf21* locus, rs9782955 in *LYST*, and rs3794060 in *NADSYN1*) (Table 1). Three of them were nonsynonymous,

Table 1. SNP information for allele genotyping

SNPs	Gene locus	Location	SNP effect	Amino acid change
rs1801274	<i>FCGR2A</i>	Chr.1:161479745	Missense variant	H131R
rs2286672	<i>PLD2</i>	Chr.17:4712617	Missense variant	R172C
rs887369	<i>CXorf21</i>	Chr.X:30577846	Synonymous variant	V209V
rs9782955	<i>LYST</i>	Chr.1:236039877	Intron	-
rs3794060	<i>NADSYN1</i>	Chr.11:71187679	Intron	-

SNP, single-nucleotide polymorphism.

Table 2. Alternative allele frequencies of five SNPs in patients with SLE and controls

SNPs	Ref./Alt.	Case	Control	1000 Genome Database					
				All	AFR	AMR	EAS	EUR	SAS
rs1801274	A/G	0.285	0.24	0.44	0.53	0.45	0.28	0.51	0.42
rs2286672	C/T	0.382	0.38	0.19	0.01	0.1	0.42	0.09	0.39
rs887369	A/C	0.968	0.979	0.9	0.92	0.92	0.94	0.76	0.95
rs9782955	T/C	0.89	0.88	0.84	0.98	0.75	0.88	0.77	0.76
rs3794060	C/T	0.38	0.38	0.35	0.14	0.45	0.38	0.7	0.15

SNP, single-nucleotide polymorphism; SLE, systemic lupus erythematosus; Ref./Alt., reference allele/alternative allele; All, average of entire population; AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian.

Table 3. Genotypic association between each SNP and SLE risk in Korean SLE patients and healthy controls

Variable		SNP ID (gene)				
		rs1801274 (<i>FCGR2A</i>)	rs2286672 (<i>PLD2</i>)	rs887369 (<i>CXorf21</i>)	rs9782955 (<i>LYST</i>)	rs3794060 (<i>NADSYN1</i>)
Ref./Alt.		A/G	C/T	A/C	T/C	C/T
AF		0.285	0.382	0.968	0.89	0.38
SLE cases	RH/HT/AH	339/260/57	155/188/60	13/0/393	12/117/525	258/298/100
	Total	656	403	406	654	656
Controls	RH/HT/AH	359/227/36	239/293/90	1/24/597	9/130/483	241/292/89
	Total	622	622	622	622	622
Allelic model	OR (95% CI)	1.26 (1.06–1.50)	1.00 (0.84–1.21)	0.65 (0.37–1.12)	1.12 (0.88–1.43)	1.01 (0.86–1.18)
	p-value	0.01	0.93	0.12	0.37	0.93
Dominant model	OR (95% CI)	1.28 (1.02–1.59)	1.00 (0.77–1.29)	0.05 (0.01–0.37)	0.79 (0.33–1.88)	0.98 (0.78–1.22)
	p-value	0.03	0.99	0.00	0.59	0.83
Recessive model	OR (95% CI)	1.55 (1.01–2.39)	1.03 (0.73–1.47)	1.27 (0.64–2.50)	1.17 (0.89–1.53)	1.08 (0.79–1.47)
	p-value	0.046	0.85	0.50	0.25	0.64

SNP, single-nucleotide polymorphism; SLE, systemic lupus erythematosus; Ref./Alt., reference allele/alternative allele; AF, alternative allele frequency; RH, number of homozygous references; HT, number of heterozygous; AH, number of homozygous alternates; OR, odds ratio; CI, confidence interval.

and the other two were intronic SNPs.

Association with SLE

A total of 656 cases and 622 controls were enrolled and genotyped to analyze the association with SLE. Minor allele frequencies of the five SNPs in this study were similar to those in East Asians from 1000 Genomes Database (Table 2) [19], suggesting that this study group can represent the Korean population and that our genotyping was reliable. We performed an association analysis with three different genetic models: allelic, dominant, and recessive (Table 3).

Of the five SNPs, rs1801274 in *FCGR2A* was significantly associated with the risk of SLE in all three models, but the other four SNPs did not show a significant association. Under allelic model, the minor allele frequency of genotype G in rs1801274 was significantly higher in SLE patients (AF = 0.285) than in controls (AF = 0.24) (OR, 1.26; 95% CI, 1.06 to 1.50; $p = 0.01$). Also, the dominant model of minor allele in rs1801274 seems significantly associated with SLE (OR, 1.28; 95% CI, 1.02 to 1.59; $p = 0.03$). Under recessive model, the genotype G/G of rs1801274 was significantly associated with SLE (OR, 1.55; 95% CI, 1.01 to 2.39; $p = 0.046$).

Discussion

SLE is a complex autoimmune disease caused by complex interactions between genetic and environmental components [4, 20]. Its high heritability and relatively higher risks in siblings suggest a genetic contribution to the risk of SLE [21, 22]. In this study, we aimed to replicate five SNPs that were discovered in Caucasians [12] but not replicated in Koreans

(rs2286672 in *PLD2* locus, rs887369 in *CXorf21* locus, rs9782955 in *LYST*, and rs3794060 in *NADSYN1*) or replicated in Koreans (rs1801274 in *FCGR2A* locus) with insufficient sample size to conclude any association. Through a replication study with 656 cases and 622 controls, rs1801274 in *FCGR2A* was found to be significantly associated with SLE in Koreans. However, the other four candidate SNPs were not replicated in Koreans.

The risk allele (G) of rs1801274 in *FCGR2A* is a missense variant that changes a histidine to arginine [23]. The human Fc γ receptor (*FCGR*) gene family (*FCGR2A*, *FCGR2B*, and *FCGR2C*) is located on chromosome 1q23, and it is considered to be associated with the susceptibility to SLE [23, 24]. Functionally, human Fc γ receptor is involved in anti-inflammatory regulation of immune responses. Another important role of human Fc γ receptor is the clearance of immune complexes [25]. Indeed, *FCGR* polymorphisms have been reported to be associated with the susceptibility to autoimmune diseases, including SLE [26–28]. A well-known polymorphism is the missense variant at amino acid 131 in *FCGR2A*, which decreases binding affinity to human immunoglobulin G [23, 28, 29]. The homozygosity for R131, being regarded as the low-binding allele of *FCGR2A*, is known to be associated with a 1.30-fold greater risk for the development of SLE compared with both of the other genotypes combined [30, 31]. Thus, *FCGR2A*-R131 may have potential roles in causing defective immune complex clearance and increased tissue deposition, thereby leading to accelerated organ damage [30, 32]. *FCGR2A* on phagocytic cells is a receptor for C-reactive protein (CRP) and the serum amyloid P (SAP) component, both acting as opsonins for nuclear antigens

[33, 34], and the binding between receptor and CRP or SAP might be influenced by polymorphisms [30, 35], because there is a dose-response characteristic between FCGR2A-R/H131 and the risk of SLE, with an escalation of SLE risk between RR homozygotes and RH heterozygotes [30-32]. Vigato-Ferreira *et al.* [27] examined the allelic distribution of FCGR2A in a Brazilian population (157 SLEs and 160 controls). They found that two polymorphisms of FCGR2A were associated with SLE (FCGR2A-R-131 and RR-131), noting that the FCGR2A-H-131 allele was associated with the susceptibility to arthritis and anti-DNA antibodies [27]. In the meta-analysis performed by Li *et al.* [28], the FCGR2A-R/H131 variant was associated with the development of SLE in Asians both at the allele and genotype level. Low copy number of FCGR genes, including FCGR2A-RR-131, FCGR3B, and the 2B.4 haplotype of FCGR2B, has also been reported to be associated with the susceptibility to SLE [26]. Other studies also reported the association between FCGR2A and SLE nephritis in the Korean population [36-39], demonstrating that the FCGR2A H131R missense variation is presented as a risk allele of SLE and SLE nephritis. However, the association was hard to conclude because of the lack of power due to the limited small sample size (73 to 300 patients).

In conclusion, in this study, we performed a replication study of five SNPs, previously known to be associated with SLE but not replicated in Koreans, with a relatively large amount of samples, demonstrating that rs1801274 in FCGR2A shows a nominally significant association with SLE, but the other four SNPs appear to have no association in the Korean population. Our result is supportive that FCGR polymorphisms may play important roles in the susceptibility to SLE in diverse populations, including Koreans.

Supplementary materials

Supplementary data including one table can be found with this article online at <http://www.genominfo.org/src/sm/gni-14-85-s001.pdf>.

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