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ORIGINAL ARTICLE

Single nucleotide polymorphisms of *CD244* gene predispose to renal and neuropsychiatric manifestations with systemic lupus erythematosus

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Abstract The objective of this study was to explore the association of single nucleotide polymorphisms (SNPs) of the *CD244* gene with several clinical features of systemic lupus erythematosus (SLE). Two hundred and forty-three patients with SLE and 369 healthy controls were enrolled. Two SNPs (rs6682654 and rs3766379) in the *CD244* gene were determined by allelic discrimination using a specific TaqMan probe. Only SNP rs3766379 was significantly associated with susceptibility to SLE [$P = 0.009$; odds ratio (OR) 1.28; 95% confidence interval (CI) 1.04–1.57]. The association was preferentially observed in subsets of SLE patients with nephritis and neuropsychiatric lupus. The frequency of the rs6682654 C allele was strongly associated with nephritis and neuropsychiatric lupus ($P = 0.00065$; OR 1.99; 95% CI 1.34–2.95, and $P = 1.6 \times 10^{-7}$; OR 3.47; 95% CI 2.12–5.70, respectively), as was the frequency of the rs3766379 T allele ($P = 0.0014$; OR 1.86; 95% CI 1.27–2.71, and $P = 2.6 \times 10^{-7}$; OR 3.15; 95% CI 2.00–4.96, respectively). In this study, an SNP of the *CD244* gene was associated with susceptibility to SLE. There was a strikingly strong association in SLE patients with nephritis and neuropsychiatric lupus, suggesting that this genetic marker could predict involvement of those severe complications.

Keywords Systemic lupus erythematosus · CD244 · Single nucleotide polymorphisms

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Introduction

Connective tissue diseases are rare disorders characterized by chronic inflammation of systemic organs due to dysregulation of the autoimmune system, and the incidence of systemic lupus erythematosus (SLE) is relatively high among the connective tissue diseases. In the past decade, susceptibility genes for these diseases were identified by many genetic studies. Genes including *STAT4*, *TRAF1-C5*, *IRF-5, IRAK1*, and *TLR5/9* were associated with susceptibility to SLE [1–6]. These genetic studies help to reveal the pathogenesis and prognosis of individuals with SLE, contributing to novel treatments that could lead to the development of personalized therapies.

CD244 belongs to a signaling lymphocyte activation molecule (SLAM) family and is expressed on all natural killer (NK), gamma delta ($\gamma\delta$), and memory CD8+ ($\alpha\beta$) T cells, whereas its ligand, CD48, is broadly expressed on all nucleated hematopoietic cells. A recent study of the molecular mechanisms of CD244 indicated its important roles in the immune system in NK and T cells [7]. It is possible that SLAM family proteins contribute to the development of autoimmune disorders, because a causal variant of a murine lupus model was identified in *Ly108*, another member of the SLAM family [8]. A family-based association study of UK and Canadian families with SLE revealed variants in the promoter and coding region of SLAMF7 and LY9 [9], which were situated adjacent to CD244. The strongest association was detected in exon 8 of LY9. These findings suggest that CD244 may be involved in susceptibility to connective tissue diseases, and a recent study reported that SNPs in *CD244* were significantly associated with susceptibility to rheumatoid arthritis (RA) and SLE in a Japanese population [10]. However, they could not replicate the association of the *LY9* gene with

susceptibility to SLE in a Japanese population, and another recent report [11] could not replicate the association in 16 collections from nine European countries.

In this study, we explored the influence of the SNPs of *CD244* on the susceptibility to SLE in a Japanese population. We also evaluated the association between the SNPs and clinical subsets of SLE.

Patients and methods

Patients

Two hundred and forty-three patients with SLE and 369 healthy controls (HC) were enrolled in this study (Table 1). All individuals were of Japanese origin. The patients with SLE fulfilled the American College of Rheumatology criteria for the classification of SLE [12]. In patients with SLE, the most common clinical manifestations in this study were nephritis (44%), cytopenia (32%), and neuropsychiatric manifestations (30%; Table 1). The prevalence of butterfly rash was 17%, arthritis was 25%, serositis was 6%, and antiphospholipid antibody syndrome was 5%. In this study, patients with overlap syndrome and mixed connective tissue disease were excluded from the analysis. Genomic DNA was obtained from all individuals enrolled after they provided informed consent. This study met the criteria of the ethics committees of Tokyo Women's Medical University.

Assessment of clinical characteristics of patients with SLE

The clinical manifestations evaluated in this study were defined according to the American Rheumatism Association Glossary Committee [13]. The patients with arthralgia and joint swelling but no joint destruction were defined as having SLE-related arthritis. The patients with serositis included pleuritis, pericarditis, and/or peritonitis. Persistent proteinuria >0.5 g daily or cellular casts identified on urinalysis were considered evidence of nephritis. SLE patients with suspected nephritis were biopsied after informed consent was obtained. Lupus nephritis included classes II–V in the World Health Organization classification. Neuropsychiatric manifestations were defined according to the American College of Rheumatology nomenclature and definitions [14]. A diagnosis of cytopenia was defined for leukopenia (white blood cell count $<4 \times 10^3/mm^3$), lymphopenia (lymphocyte <1500 per mm^3), anemia (hemoglobin levels <10 g/dl), or thrombocytopenia (platelet count $<1 \times 10^5/mm^3$) related to autoimmunity. The patients with antiphospholipid antibody syndrome fulfilled the Sapporo criteria for classification of the syndrome.

Table 1 Age and gender with systemic lupus erythematosus and healthy controls

	SLE ($n = 243$)	HC ($n = 369$)
Age, years, median (range)	34 (17–77)	32 (20–74)
Gender, female:male	231:12	349:20
Clinical features, no (%)		
Butterfly rash	42 (17)	
Arthritis	61 (25)	
Serositis	15 (6)	
Nephritis	108 (44)	
NP	72 (30)	
Cytopenia	77 (32)	
APS	13 (5)	

SLE systemic lupus erythematosus, *HC* healthy controls, *NP* neuropsychiatric-lupus, *APS* antiphospholipid antibody syndrome

Allelic discrimination of SNPs in the *CD244* gene

The SNPs in *CD244* (rs6682654 and rs3766379) were genotyped with the TaqMan SNP Genotyping Assay kit and an ABI 7900HT system (Applied Biosystems, Foster City, CA, USA). The forward and reverse primers for the polymerase chain reaction, two allele-specific oligonucleotide probes labeled with a fluorescent reporter dye (FAM or VIC), and a quencher dye, which was specific for the SNPs of the *CD244* gene, are commercially available from Applied Biosystems.

Statistical analysis

Association analyses of allele and genotype frequencies of the SNPs (rs6682654 and rs3766379) in *CD244* were conducted using the chi-square test with 2×2 and 2×3 contingency tables, with the Fisher exact test being used when appropriate. The primary analysis was performed without adjustment for multiple tests, but we also confirmed the significance by performing the analyses with Bonferroni correction. The odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the strength of the association. Differences were considered to be significant at $P < 0.05$.

Results

Association between the SNPs (rs6682654 and rs3766379) in *CD244* and susceptibility to SLE

We used the allelic discrimination method to determine genotypes of SNPs in patients with SLE and in HC. As shown in Table 2, there was a significant difference in the

Table 2 Distribution of alleles and genotypes of the single nucleotide polymorphisms in *CD244*

	rs6682654							rs3766379						
	Allele, *n* (%)				Genotype, *n* (%)			Allele, *n* (%)				Genotype, *n* (%)		
	C	T	*P*	OR (95% CI)	CC	CT	TT	*P*	T	C	*P*	OR (95% CI)	TT	TC	CC	*P*
SLE	327 (67)	159 (33)	0.021	1.21 (0.97–1.50)	110 (45)	107 (44)	26 (11)	0.067	301 (62)	185 (38)	0.009	1.28 (1.04–1.57)	92 (38)	117 (48)	34 (14)	0.029
HC	448 (61)	290 (39)			138 (37)	172 (47)	59 (16)		401 (54)	337 (46)			111 (30)	179 (49)	79 (21)	

SLE systemic lupus erythematosus, *HC* healthy controls, *OR* odds ratio, *CI* confidence interval

P values were estimated by the Fisher exact test compared with HC

frequencies of the rs3766379 alleles between patients with SLE and HC ($P = 0.009$, OR 1.28, 95% CI 1.04–1.57). The T allele was significantly more common in patients with SLE than that in HC. In contrast, there were no differences in the frequencies of the rs6682654 alleles and genotypes between patients with SLE and HC when Bonferroni correction was applied. The genotype frequencies of HC did not deviate from Hardy–Weinberg equilibrium ($P = 0.32$).

Relationship of the *CD244* SNPs to clinical features in SLE

Patients with SLE show a variety of clinical manifestations. To investigate which manifestations were associated with the SNPs, the SNP frequencies were compared between patients with and without certain clinical features, including butterfly rash, arthritis, serositis, nephritis, neuropsychiatric lupus, cytopenia, and antiphospholipid antibody syndrome. We identified no significant differences in the frequencies of alleles and genotypes between patients with versus without butterfly rash, arthritis, serositis, and antiphospholipid antibody syndrome of SLE (Table 3). In contrast, there were strikingly significant differences in the frequencies of both rs6682654 and rs3766379 alleles and genotypes between patients with and without neuropsychiatric manifestations and nephritis (Table 3). Although there was a weak correlation of the rs6682654 allele to cytopenia, the correlation disappeared when Bonferroni correction was applied. There were also significant differences in the frequencies of alleles and genotypes of the SNPs between patients with neuropsychiatric manifestations and nephritis versus HC (data not shown), and the associations remained significant when Bonferroni correction was applied.

Discussion

In this study, we replicated the weak association of the SNP rs3766379 in *CD244* with susceptibility to SLE in the Japanese population, as previously reported [10], whereas we found no significant association between the SNP rs6682654 in *CD244* and susceptibility to SLE. Recently, a big study from South Korea failed to show the significant association between two SNPs (rs3766379 and rs6682654) and susceptibility to SLE in a Korean population [15]. Those previous studies did not investigate the association between clinical phenotypes in SLE and SNPs. We demonstrated for the first time that both of these two SNPs in *CD244* were strongly associated with the clinical features of nephritis and neuropsychiatric lupus in SLE patients. These clinical features are the most severe manifestations

Table 3 Association of alleles and genotype of the single nucleotide polymorphisms in *CD244* with clinical features in patients with systemic lupus erythematosus

Clinical feature		Variable															
		rs6682654								rs3766379							
		Allele, *n* (%)				Genotype, *n* (%)				Allele, *n* (%)				Genotype, *n* (%)			
		C	T	*P*	OR (95% CI)	CC	CT	TT	*P*	T	C	*P*	OR (95% CI)	TT	CT	CC	*P*
Butterfly rash	(+)	58 (69)	26 (31)	0.798	1.10	22 (53)	14 (33)	6 (14)	0.252	55 (65)	29 (35)	0.537	1.20	20 (47)	15 (36)	7 (17)	0.19
	(−)	269 (67)	133 (33)		(0.60–1.83)	88 (44)	93 (46)	20 (10)		246 (61)	156 (39)		(0.73–1.97)	72 (36)	102 (51)	27 (13)	
Arthritis	(+)	85 (70)	37 (30)	0.578	1.16	26 (43)	33 (54)	2 (3)	0.397	78 (64)	44 (36)	0.667	1.12	22 (36)	34 (56)	5 (8)	0.2182
	(−)	242 (66)	122 (34)		(0.74–1.80)	84 (46)	74 (41)	24 (13)		223 (61)	141 (39)		(0.73–1.72)	70 (38)	83 (46)	29 (16)	
Serositis	(+)	25 (83)	5 (17)	0.069	2.55	10 (67)	5 (33)	0 (0)	0.188	24 (80)	6 (20)	0.0504	2.58	9 (60)	6 (40)	0 (0)	0.121
	(−)	302 (66)	154 (34)		(0.96–6.79)	100 (44)	102 (45)	26 (11)		277 (61)	179 (39)		(1.04–6.45)	83 (36)	111 (49)	34 (15)	
Nephritis	(+)	163 (75)	53 (25)	0.00065	1.99	65 (60)	33 (31)	10 (9)	0.000119	151 (70)	65 (30)	0.0014	1.86	56 (52)	39 (36)	13 (12)	0.000272
	(−)	164 (61)	106 (39)		(1.34–2.95)	45 (33)	74 (55)	16 (12)		150 (56)	120 (44)		(1.27–2.71)	36 (27)	78 (58)	21 (15)	
NP	(+)	121 (84)	23 (16)	1.63×10^{-7}	3.47	54 (75)	13 (18)	5 (7)	9.19×10^{-9}	114 (79)	30 (21)	2.55×10^{-7}	3.15	48 (67)	18 (25)	6 (8)	1.69×10^{-8}
	(−)	206 (60)	136 (40)		(2.12–5.70)	56 (33)	94 (55)	21 (12)		187 (55)	155 (45)		(2.00–4.96)	44 (26)	99 (58)	28 (16)	
Cytopenia	(+)	114 (74)	40 (26)	0.037	1.59	42 (55)	30 (39)	5 (6)	0.1003	105 (68)	49 (32)	0.057	1.49	36 (47)	33 (43)	8 (10)	0.138
	(−)	213 (64)	119 (36)		(1.04–2.43)	68 (41)	77 (46)	21 (13)		196 (59)	136 (41)		(0.99–2.23)	56 (34)	84 (50)	26 (16)	
APS	(+)	15 (58)	11 (42)	0.289	0.65	4 (31)	7 (54)	2 (15)	0.464	14 (54)	12 (46)	0.41	0.70	3 (23)	8 (62)	2 (15)	0.492
	(−)	312 (68)	148 (32)		(0.29–1.44)	106 (46)	100 (44)	24 (10)		287 (62)	173 (38)		(0.32–1.56)	89 (39)	109 (47)	32 (14)	

CI confidence interval, *NS* not significant, *OR* odds ratio, *NP* neuropsychiatric lupus, *APS* antiphospholipid antibody syndrome

P values of the comparison of the frequencies of allele with each clinical feature

relating to the mortality and morbidity of SLE. These findings suggest that the SNPs predict the occurrence of life-threatening complications of SLE.

A previous study [10] using a large cohort indicated that two SNPs in *CD244* were associated with susceptibility to SLE ($n = 554$) in a Japanese population, which was partially confirmed by our cohort of patients with SLE. However, we failed to replicate a significant association of the SNP at rs6682654 with susceptibility to SLE. Because our results were obtained from DNA samples from a cohort containing 243 patients with SLE and from 369 HC, the limitation in the study can be a small population of patients with SLE. Distributions of the rs6682654 genotypes in SLE were 74 (T/T), 238 (C/T), and 242 (C/C) in the previous report, and 26 (T/T), 107 (C/T), and 110 (C/C) in this study, respectively. Distributions of the rs6682654 genotypes in HC were 164 (T/T), 439 (C/T), and 336 (C/C) in the previous report, and 59 (T/T), 172 (C/T), and 138 (C/C) in this study, respectively. In summary, there were no significant differences between the two sets of patients with SLE ($P = 0.60$) and HC ($P = 0.77$) according to Fisher's exact test. We did not collaborate in the previous study at all, and it is possible that most of our samples are different from those examined in the previous study. Combined analysis of this and previous data indicates the distributions of rs3766379 genotypes are 301 (T/T), 360 (T/C), and 136 (C/C) in SLE patients, and 383 (T/T), 631 (T/C), and 293 (C/C) in HC, respectively, which showed a significant difference ($P = 7.9 \times 10^{-5}$) according to Fisher's exact test. Also, there was a significant difference in rs3766379 allele frequencies between patients with SLE and HC ($P = 1.2 \times 10^{-5}$, OR 1.3, 95% CI 1.2–1.5). The distributions of rs6682654 genotypes are 100 (T/T), 345 (C/T), and 352 (C/C) in SLE patients and 223 (T/T), 611 (C/T), and 474 (C/C) in HC. There were significant differences in both genotype ($P = 0.00038$) and allele frequencies ($P = 5.6 \times 10^{-5}$, OR 1.3, 95% CI 1.1–1.5) between patients with SLE and HC. Thus, this combined analysis using 797 patients with SLE and 1308 HC in a Japanese population indicated that the *CD244* gene is associated with susceptibility to SLE.

In conclusion, our study provided independent replication of the *CD244* gene's association with SLE using a Japanese SLE cohort. Because replication in independent studies is indispensable when considering a genetic factor, we conclude that *CD244* is a promising gene associated with susceptibility to SLE and with two specific clinical manifestations of SLE, nephritis and neuropsychiatric lupus, in a Japanese population.

Conflict of interest statement None.

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