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Concise Report

MERTK polymorphisms associated with risk of haematological disorders among Korean SLE patients

H. S. Cheong, S. O. Lee, C.-B. Choi[1], Y.-K. Sung[1], H. D. Shin and S.-C. Bae[1]

***Objective*. The MER receptor tyrosine kinase (*MERTK*) gene is critical for the efficient clearance of apoptotic cells and has implications for inflammation and autoimmune diseases such as systemic lupus erythematosus (SLE). We investigated the genetic polymorphisms in *MERTK* to evaluate it as a potential candidate gene for a host genetic study of SLE and clinical manifestations in patients with SLE.**

***Methods*. By resequencing the coding and flanking regions of the *MERTK* gene in 24 unrelated Koreans, 37 polymorphisms were identified. Based on gene position, minor allele frequency and inter-single-nucleotide polymorphism (SNP) linkage disequilibrium, six of these polymorphisms were selected for subsequent genotyping and association analysis with the risk of SLE and haematological disorders in 350 Korean SLE patients and 330 controls.**

***Results*. Although no significant associations with the risk of SLE were found, logistic regression analyses revealed that variants *+465C > G* ($P = 0.05$) and *+130215insdelT* ($P = 0.0005$) were significantly associated with decreased risk of leucopenia in SLE patients. Further, *+465C > G*, *+95616G > A*, *+123157A > G* and the haplotype *ht1* also showed significant associations ($P = 0.006$–0.05) with a decreased risk of lymphopenia in SLE patients.**

***Conclusion*. Our findings suggest that polymorphisms in *MERTK* might be one of the genetic risk factors for presenting leucopenia and lymphopenia in SLE patients.**

KEY WORDS: *MERTK*, SLE, Haematological disorder, Leucopenia, Polymorphism.

Introduction

Systemic lupus erythematosus (SLE) is characterized by the accelerated apoptosis of peripheral lymphocytes and the impaired clearance of apoptotic cells [1]. The aetiology of SLE is very complex, involving both environmental and genetic factors and probably a synergistic relationship between these factors. Genetic factors are likely to play a significant role in susceptibility to SLE and in determining the disease expression [2].

MERTK is a member of the receptor tyrosine kinase family of cell-surface receptors that includes Axl and Tyro3, and consists of an intracellular kinase-containing domain, a transmembrane region and a cell-adhesion molecule-related extracellular domain [3–5]. *MERTK* is expressed in a number of tissues including macrophages, epithelia and reproductive tissue [6]. It is required for the clearance of apoptotic cells by mononuclear phagocytes in mice, with its absence resulting in progressive lupus-like autoimmunity. Mice carrying mutations in *MERTK* have impairments in phagocytosis and clearance of apoptotic cells and develop anti-DNA autoantibodies [7, 8]. These findings provide evidence to support the concept that defects in the ability of *MERTK* to clear apoptotic cells may underlie systemic autoimmunity like SLE.

It was recently shown that polymorphisms of *MERTK* are associated with an increased risk for retinitis pigmentosa [9, 10], but no genetic study into the relationship with SLE has been reported. The aim of the present study is to investigate the role of *MERTK* polymorphisms with the risk of SLE and clinical manifestations in Korean patients with SLE.

Material and methods

Subjects

A total of 350 Korean SLE patients who fulfilled the 1997 American College of Rheumatology (ACR) criteria of SLE [11] were consecutively enrolled between September 1998 and February 2002 from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea. The following clinical and laboratory data were obtained: sex, age, ages at first symptom onset and clinical diagnosis and ACR diagnosis [12]. The clinical profiles are summarized in Table 1. The presence of four elements: haemolytic anaemia (with reticulocytosis), leucopenia ($<4000/\mu$l on two or more occasions), lymphopenia ($<1500/\mu$l on two or more occasions) and thrombocytopenia ($<100\,000/\mu$l in the absence of offending drugs) is considered as a single haematological disorder [12]. As a control group, we included 330 healthy ethnically matched subjects. Written, informed consent was obtained from each subject. The study was approved by Institutional Review Board of Hanyang University Medical Center.

Department of Genetic Epidemiology, SNP Genetics, Inc., Rm 1407, 14th floor, B-dong, WooLim Lion's Valley, 371-28, Gasan-dong, Geumcheon-Gu, Seoul 153-803 and [1]Department of Internal Medicine, Division of Rheumatology, the Hospital for Rheumatic Diseases, Hanyang University, Seoul 133-792, Korea.

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Correspondence to: Sang-Cheol Bae, MD, PhD, MPH, the Hospital for Rheumatic Diseases, Hanyang University Medical Center, 17 Haengdang-Dong, Seongdong-Gu, Seoul, Korea 133-792. E-mail: scbae@hanyang.ac.kr

Table 1. Clinical profiles of the study subjects

Criteria		Description	
		SLE patients	Normal controls
Number of subjects		350	330
Age [mean (range)]		35.6 (10.5–72.7)	35.6 (20.6–71.5)
Sex (male/female)		15/335	46/284
Disease duration [mean (range)]		29.1 (5.3–63.7)	–
		Positive rates	
ACR[a]	Butterfly rash	34.80%	0%
	Discoid lupus	4.90%	0%
	Photosensitivity	27.20%	0%
	Oral ulcer	34.50%	0%
	Arthritis	64.10%	0%
	Serositis	26.70%	0%
	Renal disorder	36.20%	0%
	Neurological disorder	5.80%	0%
	Hematological disorder	71.00%	0%
	Immunological disorder	80.00%	0%
	Antinuclear Ab	100.00%	0%

[a]1997 ACR (American College of Rheumatology) criteria for SLE.

Sequencing analysis of MERTK

We resequenced the entire coding region along with 1.5 kb of the promoter region of the *MERTK* gene, in DNA samples from 24 unrelated, healthy Koreans using a DNA analyzer (ABI PRISM 3730, Applied Biosystems, Foster City, CA, USA). Twenty-three polymerase chain reaction (PCR) primer sets were designed based on the GenBank reference sequence (NT_022135). Information regarding the primers used is available on our website (http://www.snp-genetics.com/user/additional_list.asp). Sequence analysis was carried out using SeqMan® software.

Genotyping with fluorescence polarization detection

For genotyping of polymorphic sites, amplifying primers and probes were designed for TaqMan® [13]. Primer Express (Applied Biosystems) was used to design both the PCR primers and the minor groove binder (MGB) TaqMan probes. One allelic probe was labelled with the 6-carboxyfluorescein (FAM) dye and the other with the fluorescent VIC dye. Typically, PCR was run in the TaqMan Universal Master mix without uracil-DNA glycosylase (UNG) (Applied Biosystems) at primer concentration of 900 nM and TaqMan MGB probe concentration of 200 nM. The reaction was performed in a 384-well format in a total reaction volume of 5 μl using 20 ng of genomic DNA. The plate was then placed in a thermal cycler (PE 9700, Applied Biosystems), and heated for 2 min at 50°C and for 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan assay plate was then transferred to a Prism 7900HT instrument (Applied Biosystems), where the fluorescence intensity of each well was read. Fluorescence data files from each plate were analysed by automated software (SDS 2.1). Detailed information concerning the primers can be obtained at the web site aforementioned.

Statistics

We examined Lewontin's D′ (|D′|) and the linkage disequilibrium coefficient R^2 between all pairs of biallelic loci [14]. Haplotypes of each individual were inferred using the algorithm developed by Schaid *et al.* [15] (haplo.score). The genotype distributions of *MERTK* polymorphisms comparing the SLE patients and normal subjects, and among SLE patients, were analysed with logistic regression models while controlling for age (continuous variable), sex (male = 0, female = 1) and/or disease duration (continuous variable) as covariates. Genetic effects of inferred haplotypes were analysed using haplo.score while controlling for the same three covariates.

Results

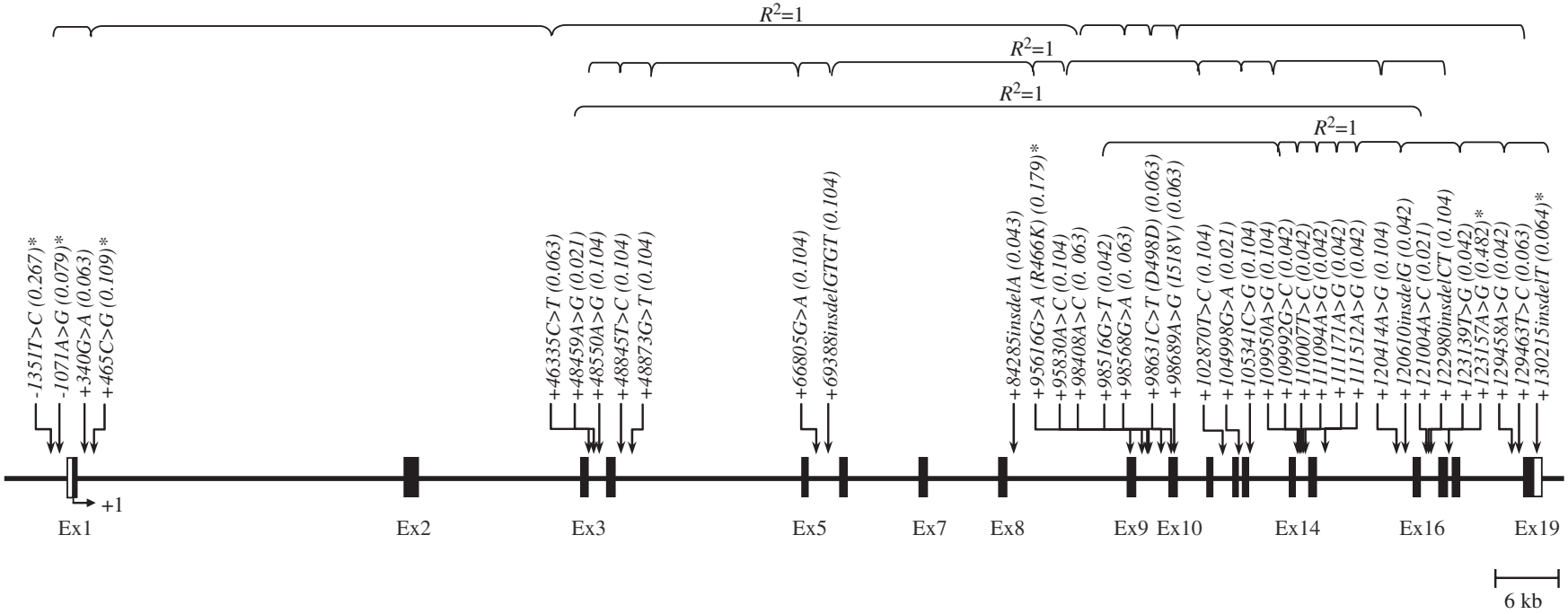
By direct sequencing of 24 individuals, we identified 37 sequence variants in the *MERTK* gene: two in the promoter, three in coding regions of exons, 31 in introns and 1 in the 3′untranslated region (UTR) (Fig. 1). Pair-wise comparisons among all 37 polymorphisms revealed four sets of markers in absolute linkage disequilibrium with each other ($|D'| = 1$ and $R^2 = 1$, Fig. 1A). Genotype distributions of all 37 polymorphisms were in Hardy–Weinberg equilibrium (HWE) ($P > 0.05$) (Table 2). Among this group of 37 variants, 6 common ones [*−1351T > C*, *−1071A > G*, *+465C > G*, *+95616G > A* (*Arg466Lys*), *+123157A > G* and *+130215insdelT*] were selected for genotyping based on location (polymorphisms in exons were preferred), minor allele frequency (MAF) exceeding 0.05 and LD [only one polymorphism was chosen if it was in absolute LD ($R^2 = 1$) with one or more other polymorphisms]. The MAFs of the six genotyped polymorphisms were 0.267 (*−1351T > C*), 0.079 (*−1351T > C*), 0.109 (*+465C > G*), 0.179 (*+95616G > A*), 0.482 (*+123157A > G*) and 0.064 (*+130215insdelT*) (Fig. 1A). Pair-wise comparisons among these six polymorphisms revealed complete LD in several cases ($|D'| = 1$ and $R^2 \neq 1$) (Fig. 1C). Only two common haplotypes (*ht1, ht2*) had frequencies greater than 0.01, and both were used for haplotype association analysis (Fig. 1B).

The genotype distributions of *MERTK* polymorphisms among SLE patients and normal subjects were analysed with logistic regression models. No significant genetic associations were found between *MERTK* polymorphisms and the risk of SLE (Table 3). Genetic associations with the risk of haematological disorders (haemolytic anaemia, leucopenia, lymphopenia and thrombocytopenia) were also tested. Logistic regression analyses revealed that *+465C > G* and *+130215insdelT* were significantly associated ($P = 0.05$ and 0.0005, respectively) in a dominant model with a decreased risk of leucopenia in SLE patients. *+465C > G*, *+95616G > A*, *+123157A > G* and the haplotype *ht1* (*TACGAdel*) also showed significant associations with the decreased risk of lymphopenia in SLE patients ($P = 0.03$, 0.03, 0.05 and 0.03, respectively) (Table 4). The association of haplotype *ht1* was attributed to *+123157A > G* because *ht1* was almost completely tagged by *+123157A > G* (Fig. 1B).

Discussion

Like other autoimmune diseases, SLE shows a strong genetic predisposition. This disease shows a high concordance in identical

(A) Map of *MERTK* (MER tyrosine kinase protooncogene) on chromosome 2q14.1



(B) Haplotypes in *MERTK*

Hap.	*-1351T>C*	*-1071A>G*	*+465C>G*	*+95616G>A*	*+123157A>G*	*+130215insdelT*	Freq.
ht1	T	A	C	G	A	del	0.492
ht2	C	A	C	G	G	del	0.255
ht3	T	G	C	A	G	del	0.077
ht4	T	A	G	A	G	ins	0.059
ht5	T	A	C	G	G	del	0.040
ht6	T	A	G	A	G	del	0.031
ht7	T	A	G	G	A	del	0.021
Others	.	.	.	.	.	.	0.027

(C) LDs among *MERTK* polymorphisms

		|D′|					
		-1351T>C	*-1071A>G*	*+465C>G*	*+95616G>A*	*+123157A>G*	*+130215insdelT*
R^2	*-1351 T>C*	-	1	1	0.905	0.951	1
	-1071 A>G	0.031	-	1	0.977	0.963	1
	+465C>G	0.045	0.011	-	0.768	0.634	0.895
	+95616 G>A	0.065	0.371	0.336	-	1	0.887
	+123157 A>G	0.356	0.085	0.054	0.237	-	1
	+130215insdelT	0.025	0.006	0.446	0.249	0.075	-

FIG. 1. Gene map, haplotypes, and LD coefficients among *MERTK* polymorphisms. **(A)** Coding exons are marked by black blocks, and 5′ and 3′ UTRs by white blocks. The first base of the translational start site is denoted as nucleotide +1. Asterisks indicate six polymorphisms that were genotyped in 350 SLE cases and 330 controls. **(B)** Haplotypes of *MERTK*. Only those with frequencies greater than 0.02 are shown. 'Others' category contains rare haplotypes: *TACAGdel*, *CACGAdel*, *TACGGins*, *CACAGdel* and *TGCGAdel*. **(C)** Linkage disequilibrium coefficients (|D′| and R^2) among *MERTK* polymorphisms.

TABLE 2. *MERTK* allele and haplotype frequencies and tests for association in Korean SLE subjects compared with normal controls

Locus	Frequency		OR (95%CI)[c]	*P*-value
	SLE ($n=350$[a])	NC[b] ($n=330$)		
−1351T > C	0.268	0.267	1.00 (0.78–1.29)	1.00
−1071A > G	0.078	0.079	0.99 (0.66–1.49)	0.97
+465C > G	0.111	0.107	1.05 (0.73–1.52)	0.78
+95616G > A	0.177	0.182	0.97 (0.73–1.29)	0.82
+123157A > G	0.486	0.479	1.02 (0.82–1.28)	0.86
+130215insdelT	0.072	0.056	1.29 (0.82–2.03)	0.28
Haplotype ht1	0.491	0.482	1.04 (0.83–1.30)	0.77
Haplotype ht2	0.258	0.260	0.97 (0.74–1.26)	0.78

[a]*n* is the number of individuals examined.
[b]NC, normal controls.
[c]Odds ratios with 95% confidence intervals [OR (95% CI)] and *P*-values were calculated using logistic regression. Age, disease duration and sex were included as covariates.

twins and occurs with an increased frequency among first-degree relatives of patients. An analysis of multiplex families suggested that the genetic component of disease susceptibility involves a set of unlinked genes that operate in concert to induce either SLE or other autoimmune diseases [16].

We examined *MERTK* as one of the promising candidate genes in the development of SLE. The results show that the polymorphisms in *MERTK* are not associated with the risk of SLE. The reason of discrepancy can be accounted for in many aspects. Firstly, in a mouse model involving a defect in an apoptosis-inducing system, abnormal apoptosis showed clear association with development of lupus [8], but it is less evident in humans [17]. Secondly, it was demonstrated that environmental adjuvants were necessary together with apoptotic cells to induce autoimmunity [18] and it would be reasonable to think that other factors are also imperative in humans. Thirdly, functional evidence of *MERTK* biology on the risk of SLE has not been clarified. It has been suggested that *MERTK* binds to phosphaditylserine, which conveys the apoptotic signals to phagocytic cells, via the protein encoded by the growth-arrest-specific gene 6 (*GAS6*). However, molecules such as C1q, β2-glycoprotein I, pentraxin 3, C-reactive protein, serum amyloid P-protein, thrombospondin, prothrombin and protein-S also play an important role in bridging inadequate removal of apoptotic cells in SLE [19, 20]. Lack of precise knowledge on *MERTK* function may have resulted in the lack of association with susceptibility to SLE.

Nevertheless, polymorphisms in *MERTK* were significantly associated with the risk of haematological disorders among SLE patients. The associations of *+465C > G* and *+130215insdelT* with leucopenia, and *+465C > G*, *+95616G > A*, *+123157A > G* and *ht1* with lymphopenia were detected in Korean SLE patients. To our knowledge, this is the first study to examine the relationship between genetic polymorphisms in *MERTK* and haematological disorders. Leucopenia occurs in 18–50% of SLE patients [21–24]. Circulating neurophils and/or lymphocytes may be depressed for a variety of reasons. Lymphopenia in SLE patients is also common and may be of pathogenic significance. Lymphopenia can occur independently of leucopenia. Lymphocytotoxic antibodies and lymphocyte apoptosis are possible causes of lymphopenia in SLE patients [25, 26]. But, as is the case with leucopenia, lymphopenia may be caused by factors other than SLE.

Haematological disorders (haemolytic anaemia, leucopenia, lymphopenia and thrombocytopenia) are common features of SLE [27, 28]. In a study of German patients with SLE, haemolytic anaemia and lymphopenia predicted lupus flares [29]. These studies support the importance of haematological disorders in the diagnosis and prognosis of SLE [24, 28–30]. Haematological disorders were not different among ethnic groups [21, 31], and several studies have shown that genetic factors are associated with haematological disorders [32, 33]. But the prevalence of these haematological disorders is not well-established.

The first *MERTK* mutation identified, R722X (a premature stop codon) was predicted to result in loss of function due to truncation or absence of the protein [10]. Another missense mutation, R884C that results in severe retinal degeneration with childhood onset when in compound heterozygous form with a R722X allele was also predicted to result in a loss of signalling of *MERTK* [9]. These two mutations were described in people of European ancestory, but were not discovered in our resequence analysis of 24 Korean individuals. Therefore, we did not test the association of these polymorphisms in Korean SLE patients.

The genetic effects of *MERTK* polymorphisms on the risk of haematological disorders were not dramatic, i.e. associated *P*-values were not significant after correction for multiple comparisons (16 tests = 2 traits × 8 polymorphisms). And the functional evidence involved in the haematological disorder mediated by alternative genotypes in intron and 3′UTR polymorphisms is not currently understood. In the case of preliminary results like this study, further replication in other cohorts is of paramount interest, because prior probabilities of true association are low, and thus, all genetic associations demand a high level of proof [34]. Further biological and/or functional evidence is also needed to confirm the suggestive associations of *MERTK* polymorphisms with the risk of haematological disorders in Korean SLE patients.

The authors have declared no conflicts of interest.

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TABLE 3. *MERTK* genoytpe frequencies and association analyses stratified by leucopenia and lymphopenia status among Korean SLE patients

		Leucopenia					Lymphopenia				
				P-value					*P*-value		
Locus	Genotype	Positive ($n=155$[a])	Negative ($n=149$)	Co-dominant	Dominant	Recessive	Positive ($n=134$)	Negative ($n=170$)	Co-dominant	Dominant	Recessive
−1351T > C	*T*	77 (50.7%)	83 (56.9%)				69 (52.3%)	91 (54.8%)			
	CT	66 (43.4%)	50 (34.3%)	0.57	0.23	0.36	59 (44.7%)	57 (38.6%)	0.50	0.60	0.08
	C	9 (5.9%)	13 (8.9%)				4 (3.0%)	18 (7.2%)			
−1071A > G	*A*	130 (85.0%)	124 (84.4%)				114 (86.4%)	140 (83.3%)			
	AG	22 (14.4%)	22 (15.0%)	0.88	0.91	0.84	17 (12.9%)	27 (16.1%)	0.37	0.37	0.79
	G	1 (0.7%)	1 (0.7%)				1 (0.8%)	1 (0.6%)			
+465C > G	*C*	123 (82.0%)	103 (72.0%)				111 (84.7%)	115 (71.0%)			
	CG	25 (16.7%)	38 (26.6%)	0.07	**0.05**	0.94	17 (13.0%)	46 (28.4%)	**0.03**	**0.006**	0.27
	G	2 (1.3%)	2 (1.4%)				3 (2.3%)	1 (0.6%)			
+95616G > A	*G*	105 (70.5%)	88 (61.1%)				96 (73.9%)	97 (59.5%)			
	AG	40 (26.9%)	49 (34.0%)	0.08	0.10	0.35	29 (22.3%)	60 (36.8%)	**0.03**	**0.01**	0.90
	A	4 (2.7%)	7 (4.9%)				5 (3.8%)	6 (3.7%)			
+123157A > G	*A*	42 (27.1%)	33 (22.3%)				36 (26.9%)	39 (23.1%)			
	AG	80 (51.6%)	74 (50.0%)	0.19	0.37	0.22	74 (55.2%)	80 (47.3%)	**0.05**	0.44	**0.02**
	G	33 (21.3%)	41 (27.7%)				24 (17.9%)	50 (29.6%)			
+130215insdelT	*del*	143 (92.3%)	115 (77.2%)				118 (88.1%)	140 (82.4%)			
	insdel	11 (7.1%)	34 (22.8%)	**0.001**	**0.0005**	–	15 (11.2%)	30 (17.7%)	0.27	0.18	–
	ins	1 (0.7%)	0 (0.0%)				1 (0.8%)	0 (0.0%)			
Haplotype ht1	*−/−*	35 (23.8%)	37 (25.9%)				22 (17.2%)	50 (30.9%)			
	−/ht1	73 (49.7%)	74 (51.8%)	0.54	–	–	72 (56.3%)	75 (46.3%)	**0.03**	–	–
	ht1/ht1	39 (26.5%)	32 (22.4%)				34 (26.6%)	37 (22.8%)			
Haplotype ht2	*−/−*	69 (52.6%)	78 (57.8%)				62 (53.0%)	85 (57.0%)			
	−/ht2	56 (42.8%)	44 (32.6%)	0.97	–	–	51 (43.6%)	49 (32.9%)	0.63	–	–
	ht2/ht2	6 (4.6%)	13 (9.6%)				4 (3.4%)	15 (10.1%)			

[a]n is the number of individuals examined.

Genotype frequencies were analysed under co-dominant, dominant and recessive genetic models using logistic regression. Haplotype frequencies were analysed assuming a co-dominant genetic model using haplo.score [22]. See Table 2 for covariates. Bold values indicate $P < 0.005$.

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