Association of a Functional Polymorphism in the *IRF5* Region With Systemic Sclerosis in a Japanese Population

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Objective. Interferon regulatory factor 5, an established susceptibility factor for systemic lupus erythematosus (SLE), plays a role in type I interferon and proinflammatory cytokine induction. A recent study showed association of a functional single-nucleotide polymorphism (SNP) in intron 1 of *IRF5*, rs2004640, with systemic sclerosis (SSc) in a European French population. We undertook the present study to determine whether *IRF5* polymorphisms are also associated with a predisposition to SSc in Japanese.

Methods. A case–control association study was performed for rs2004640 as well as for rs10954213 and rs2280714, all of which were previously reported to be associated with SLE, in 281 SSc patients and 477 healthy controls. Patients with SSc complicated by SLE or Sjögren's syndrome were excluded. Association of the rs2280714 genotype with messenger RNA (mRNA) levels of *IRF5* and adjacently located transportin 3 (*TNPO3*) was examined using the GENEVAR database.

Results. All 3 SNPs were significantly associated with SSc, with the rs2280714 A allele having the strongest association (allele frequency $P = 0.0012$, odds ratio 1.42 [95% confidence interval 1.15–1.75]). Association was preferentially observed in subsets of patients with diffuse cutaneous SSc (dcSSc) and anti–topoisomerase I antibody positivity. Conditional analysis revealed that rs2280714 could account for most of the association of these SNPs, while an additional contribution of rs2004640 was also suggested for dcSSc. The genotype of rs2280714 was strongly associated with *IRF5* mRNA expression, while only marginal association was detected with *TNPO3* mRNA expression.

Conclusion. Association of *IRF5* with SSc was replicated in a Japanese population. Whether the causal SNP is different among populations requires further investigation.

Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by tissue fibrosis of the skin and internal organs. SSc is subdivided into 2 subsets, limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). Epidemiologic data suggest a role of genetic factors, and association of polymorphisms with susceptibility or clinical characteristics has been reported (1).

A recent study demonstrated an interferon (IFN) signature in peripheral blood cells from SSc patients and supported the role of the type I IFN pathway in the pathogenesis of the disease (2). Polymorphisms of genes involved in IFN signaling are associated with systemic lupus erythematosus (SLE) (3). Among the IFN-related genes, *IRF5* has the most well-established association with SLE (4–8). IFN regulatory factor 5 (IRF-5) plays a role in the Toll-like receptor signaling pathway, acting as a master transcription factor in the activation of genes for type I IFN as well as for proinflammatory cytokines (3).

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Initially, a common haplotype of *IRF5* composed of single-nucleotide polymorphisms (SNPs) rs2004640 (exon-intron border of exon 1B) and rs2280714 (5 kb downstream of the 3'-untranslated region [3'-UTR]) was found to be associated with SLE. The rs2004640 T allele creates a 5' splice donor site, allowing the expression of *IRF5* isoforms containing exon 1B, and the rs2280714 A allele is associated with overexpression of messenger RNA (mRNA) of *IRF5* (4). Subsequently, the exon 6 polymorphism (which encodes an insertion/deletion of 10 amino acids) and the exon 9 SNP rs10954213 (which localizes to the 3'-UTR and disrupts the polyadenylation signal) were identified, and the risk haplotype in Caucasians was shown to contain the rs2004640 T allele, the exon 6 insertion, and the rs10954213 A allele (5).

In a previous study, we showed that *IRF5* is associated with SLE in Japanese and that the allele frequencies and haplotype structures of *IRF5* differed substantially between the Japanese and Caucasian populations, with population-specific SNPs appearing to play a role (7). Recently, association of *IRF5* rs2004640 with SSc in a European French population was reported (9). We performed a case–control study to determine whether *IRF5* is also associated with SSc in Japanese.

PATIENTS AND METHODS

Patients and controls. A case–control association study was performed with 281 patients (27 men and 254 women, mean ± SD age 43.0 ± 12.8 years) and 477 controls (228 men and 249 women, mean ± SD age 34.1 ± 11.3 years) recruited at the University of Kanazawa, the University of Tokyo, and the Institute of Rheumatology, Tokyo Women's Medical University. All patients and controls were unrelated Japanese. All patients fulfilled the criteria for SSc proposed by the American College of Rheumatology (formerly, the American Rheumatism Association) (10) and were classified as having either dcSSc (n = 142) or lcSSc (n = 139) according to the classification described by LeRoy and coworkers (11). Anticentromere antibody (ACA) positivity was determined by the presence of a discrete speckled pattern on indirect immunofluorescence using HEp-2 cells and was confirmed by enzyme-linked immunosorbent assay (ELISA) using recombinant human CENP-B (Medical and Biological Laboratories, Nagoya, Japan). Anti–topoisomerase I (anti–topo I) antibody levels were determined using ELISA (Medical and Biological Laboratories), and the specificity was confirmed by immunoprecipitation. Eighty-seven patients were positive for anti–topo I antibodies, and 91 were positive for ACAs.

Because the association of *IRF5* with SLE and primary Sjögren's syndrome (SS) had already been reported (3–8), patients with SSc complicated by these conditions were excluded. The sample size of this study provided detection powers of 0.95 and 0.64 for a susceptibility gene with the genotype relative risk of 1.5 and 1.3, respectively, when the risk allele frequency was 0.3 (rs2004640) and detection powers of 0.97 and 0.69 for a susceptibility gene with the genotype relative risk of 1.5 and 1.3, respectively, when the risk allele frequency was 0.5 (rs10954213 and rs2280714) (12).

This study was reviewed and approved by the research ethics committees of the University of Tsukuba, the University of Tokyo, the University of Kanazawa, and Tokyo Women's Medical University. Informed consent was provided by all donors.

Genotyping. SNPs rs729302 A>C, rs2004640 G>T, rs3807306 C>A, rs10954213 G>A, and rs2280714 A>G were genotyped using the TaqMan genotyping system (ABI 7300; Applied Biosystems, Foster City, CA). Genotyping of rs6953165 C>G, rs2004640 G>T, rs41298401 C>G, rs10954213 G>A, and rs2280714 A>G was performed by sequencing on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) in a proportion of samples. The intron 1 primers 5'-CACCGCAGACAGGTGGG-3' (forward) and 5'-GGGAGGCGCTTTGGAAGT-3' (reverse) were used for rs6953165, rs2004640, and rs41298401; the 3'-UTR primers 5'-CCCTGATTTCCCTGGTTTG-3' (forward) and 5'-AGCCAGCCAGGTGAGTGTT-3' (reverse) were used for rs10954213; and 5'-GCTGCAATTGGAAGAAGAGGG-3' (forward) and 5'-TGATGTGGATTGGAAGTGGA-3' (reverse) were used for rs2280714. The results from TaqMan genotyping were confirmed to be identical to those from direct sequencing.

Association of the rs2280714 genotype with mRNA expression levels. Association of the rs2280714 genotype with mRNA expression levels of *IRF5* and the adjacently located transportin 3 (*TNPO3*) was examined using the HapMap database (http://www.hapmap.org/index.html.en) and the GENEVAR database at the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/humgen/genevar/) as previously described (7). Normalized mRNA data from lymphoblastoid cell lines from 43 Japanese in Tokyo, Japan, and 45 Han Chinese in Beijing, China, in the HapMap database were obtained from the GENEVAR database. Association between the rs2280714 genotype and mRNA levels of *IRF5* and *TNPO3* was analyzed using simple regression analysis.

Statistical analysis. Association analyses were conducted by chi-square test. Because this study aimed to test the specific hypothesis of whether association of *IRF5* with SSc was replicated in Japanese, correction for multiple comparisons was not applied. Conditional logistic regression analysis was conducted to examine the effect of each SNP on the susceptibility to SSc after controlling for the genotype of another SNP.

Calculation of linkage disequilibrium (LD) parameters (D' and r^2) from the genotypes of 477 healthy controls was performed using Haploview version 4.0 (Broad Institute, Cambridge, MA) (http://www.broad.mit.edu/mpg/haploview/index.php). Using Haploview version 4.0, a permutation test (1 million permutations) was performed to obtain experiment-wide *P* values of the single SNPs (rs2004640, rs10954213, and rs2280714) and of haplotypes consisting of them.

RESULTS

To determine whether the association of *IRF5* polymorphisms with SSc could be replicated in a Japa-

Table 1. Association of *IRF5* region SNPs with SSc in a Japanese population*

SNP, subject subset	No. (%) with genotype†				Risk allele frequency‡	Allelic association§		Dominant association§		Recessive association§	
	1/1	1/2	2/2	Total		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs2004640											
SSc	123 (44)	117 (42)	41 (14)	281	0.35	1.27 (1.02–1.59)	0.032	1.23 (0.91–1.65)	0.18	1.72 (1.10–2.24)	0.018
dcSSc	61 (43)	54 (38)	27 (19)	142	0.38	1.43 (1.08–1.88)	0.012	1.27 (0.87–1.85)	0.22	2.37 (1.42–3.96)	0.00096
lcSSc	62 (45)	63 (45)	14 (10)	139	0.33	1.13 (0.85–1.51)	0.40	1.19 (0.81–1.73)	0.38	1.13 (0.60–2.13)	0.71
Anti–topo I positive	33 (38)	38 (44)	16 (18)	87	0.40	1.56 (1.12–2.18)	0.0081	1.56 (0.98–2.49)	0.061	2.27 (1.23–4.20)	0.0086
ACA positive	44 (48)	37 (41)	10 (11)	91	0.31	1.06 (0.75–1.49)	0.74	1.02 (0.65–1.60)	0.93	1.25 (0.60–2.58)	0.55
Control	233 (49)	201 (42)	43 (9)	477	0.30	–	–	–	–	–	–
rs10954213											
SSc	80 (28)	131 (47)	70 (25)	281	0.52	1.30 (1.05–1.60)	0.014	1.41 (1.01–1.96)	0.042	1.38 (0.98–1.93)	0.063
dcSSc	46 (32)	60 (42)	36 (25)	142	0.54	1.39 (1.07–1.81)	0.015	1.38 (0.90–2.10)	0.14	1.66 (1.10–2.50)	0.016
lcSSc	34 (24)	71 (51)	34 (24)	139	0.50	1.21 (0.92–1.58)	0.17	1.44 (0.94–2.22)	0.094	1.12 (0.72–1.74)	0.62
Anti–topo I positive	27 (31)	35 (40)	25 (29)	87	0.51	1.27 (0.92–1.75)	0.15	1.16 (0.70–1.92)	0.56	1.56 (0.94–2.57)	0.083
ACA positive	25 (27)	43 (47)	23 (25)	91	0.51	1.26 (0.92–1.73)	0.15	1.38 (0.83–2.30)	0.21	1.31 (0.79–2.18)	0.30
Control	107 (22)	218 (46)	152 (32)	477	0.45	–	–	–	–	–	–
rs2280714											
SSc	99 (35)	136 (48)	46 (16)	281	0.59	1.42 (1.15–1.75)	0.0012	1.72 (1.18–2.50)	0.0047	1.48 (1.08–2.04)	0.015
dcSSc	59 (42)	63 (44)	20 (14)	142	0.64	1.70 (1.30–2.23)	0.00013	2.05 (1.23–3.41)	0.0056	1.94 (1.32–2.85)	0.00080
lcSSc	40 (29)	73 (53)	26 (19)	139	0.55	1.18 (0.91–1.55)	0.22	1.46 (0.91–2.34)	0.12	1.10 (0.72–1.68)	0.65
Anti–topo I positive	34 (39)	41 (47)	12 (14)	87	0.63	1.62 (1.17–2.26)	0.0041	2.10 (1.12–3.95)	0.021	1.75 (1.09–2.81)	0.020
ACA positive	28 (31)	44 (49)	18 (20)	91	0.56	1.21 (0.88–1.66)	0.25	1.34 (0.77–2.34)	0.30	1.23 (0.75–2.01)	0.40
Control	128 (27)	229 (48)	120 (25)	477	0.51	–	–	–	–	–	–

* SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; anti–topo I = anti–topoisomerase I; ACA = anticentromere antibody.
† For the single-nucleotide polymorphism (SNP) rs2004640, 1 = the G allele and 2 = the T allele; for SNPs rs10954213 and rs2280714, 1 = the A allele and 2 = the G allele.
‡ For SNP rs2004640, T = the risk allele; for SNPs rs10954213 and rs2280714, A = the risk allele.
§ Odds ratios (ORs), 95% confidence intervals (95% CIs), and *P* values were calculated by chi-square test using 2 × 2 contingency tables.

nese population, we genotyped 3 SNPs: rs2004640, rs10954213, and rs2280714. The intron 1 SNP rs2004640 has been shown to be associated with SSc in a European

Figure 1. Association of the rs2280714 genotype with levels of mRNA for *IRF5* and *TNPO3*. The rs2280714 genotype was strongly associated with *IRF5* mRNA, but only marginally associated with *TNPO3* mRNA. Simple regression analyses were performed based on the mRNA expression profiling data in the B cell lines from HapMap subjects (obtained from the GENEVAR database).

French population (9), SNP rs10954213 directly affects the *IRF5* mRNA level by altering the polyadenylation signal, and SNP rs2280714, located 5 kb downstream of the 3'-UTR of *IRF5*, has also been shown to be associated with *IRF5* mRNA expression (3–5). SNP rs10954213 was in absolute LD with the previously reported susceptibility SNP rs11770589 ($r^2 = 1$, $D' = 1$) and also efficiently tagged the exon 6 insertion/deletion in Japanese ($r^2 = 0.98$, $D' = 0.99$) (7). Deviation from Hardy-Weinberg equilibrium was not observed for any of the SNPs in the control samples.

The genotyping data are shown in Table 1. The association of rs2004640 with SSc was replicated in our Japanese patients. Similar to the European data, the association was compatible with the recessive model and was observed in dcSSc but not in lcSSc.

Interestingly, the other 2 SNPs, especially rs2280714, showed a stronger association with SSc. With respect to the autoantibody profile, rs2004640 and rs2280714 exhibited a significant association in the anti–topo I antibody–positive subset of patients, but none of the SNPs showed an association in the ACA-positive subset of patients.
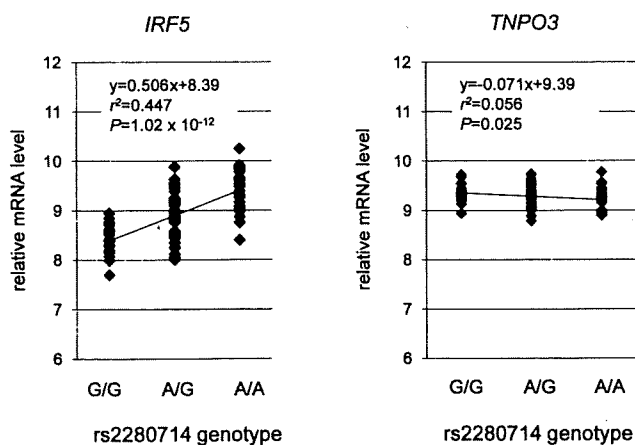
To exclude the possibility that other SNPs might

Table 2. Logistic regression analysis of the 3 SNPs examined*

Group, SNP	Model	r^2†		P‡	P§		
		With rs2004640	With rs2280714		Adjusted for rs2280714	Adjusted for rs10954213	Adjusted for rs2004640
All SSc patients							
rs2004640	Additive	NA	0.26	0.035	0.58	0.18	NA
rs10954213	Additive	0.14	0.77	0.018	0.34	NA	0.089
rs2280714	Additive	0.26	NA	0.0014	NA	0.018	0.014
All dcSSc patients							
rs2004640	Recessive	NA	0.26	0.00093	0.033	0.0058	NA
rs10954213	Additive	0.14	0.77	0.020	0.051	NA	0.15
rs2280714	Additive	0.26	NA	0.00018	NA	0.00043	0.0058

* NA = not applicable (see Table 1 for other definitions).
† Pairwise linkage disequilibrium as measured by r^2 in the controls.
‡ P values for each SNP under the recessive, additive, or dominant model that provided the best fit by logistic regression analysis.
§ P values adjusted for each SNP under the indicated model.

exhibit stronger associations, we additionally genotyped SNPs that have previously been implicated in auto-immune diseases, rs729302 A>C, rs6953165 C>G, rs41298401 C>G, and rs3807306 C>A, in 106 patients and 290 controls. None showed significant association (data not shown).

Because LD was present among SNPs rs2004640, rs10954213, and rs2280714, the contribution of each SNP was examined using conditional logistic regression analysis. In the group of all patients with SSc, conditioning by rs2280714 eliminated the significant association of other SNPs, while the association of rs2280714 remained significant after conditioning by rs10954213 or rs2004640 (Table 2). These results indicated that rs2280714 accounts for the genetic effect of the IRF5 region in SSc. When the same analysis was used for the subgroup of patients with dcSSc, the results were essentially the same, except that the association of rs2004640 remained marginally significant after conditioning by rs2280714 (Table 2).

A permutation test demonstrated that the most significant association was observed when the rs2280714 A allele was used as a single marker (permutated $P = 0.0076$ for total SSc, permutated $P = 0.0016$ for dcSSc) rather than when the haplotype containing each susceptibility allele of the 3 examined SNPs was considered (permutated $P = 0.097$ for total SSc, permutated $P = 0.21$ for dcSSc). This further supported the significance of rs2280714 in the Japanese population.

SNP rs2280714 is located in the intergenic region between IRF5 and TNPO3. To gain insight into the functional significance of rs2280714, we investigated whether this SNP was associated with the expression of IRF5 and TNPO3 using the mRNA expression profiling

data of the B cell lines from HapMap subjects. As shown in Figure 1, the IRF5 mRNA level was strongly correlated with the number of A alleles ($r^2 = 0.447$, $P = 1.02 \times 10^{-12}$, slope = 0.506), while the rs2280714 genotype was only marginally associated with TNPO3 mRNA ($r^2 = 0.056$, $P = 0.025$, slope = -0.071).

DISCUSSION

In this study, we replicated the association of IRF5 with SSc in a Japanese population. In addition to the originally reported rs2004640 T allele (9), we also detected an association of the rs2280714 A allele and the rs10954213 A allele with SSc. SNP rs2280714 appeared to account for most of the genetic effects of this region, but an additional effect was suggested for the rs2004640 T allele in the subset of patients with dcSSc. Similar to findings in the European study, the association of IRF5 was observed in dcSSc but not in lcSSc. Furthermore, the association was observed in the anti–topo I antibody–positive subset of patients, but not in the ACA–positive subset of patients. The risk allele of rs2280714 was associated with a higher IRF5 mRNA level in an allele number–dependent manner. Taken together with its functional relevance and association with susceptibility to SLE, rheumatoid arthritis, SS, and inflammatory bowel disease in multiple populations (3–8), IRF5 is postulated to be a common genetic factor for autoimmunity.

An "IFN signature" has been shown in the expression profiling of SSc (2). Our findings not only support the role of type I IFN in the pathogenesis of SSc, but also suggest that IRF-5 overexpression may play a causal role in a proportion of patients. Of interest,

treatment with type I IFN has been suggested to trigger the development of SSc in patients with multiple sclerosis (13). If such a hypothesis is proven, type I IFN should be considered as a potential therapeutic target for SSc.

Unexpectedly, the primary role was detected for rs2280714 (located 5 kb downstream of the 3′-UTR of *IRF5*) rather than for rs2004640 (which creates the intron 1 splice donor site and is associated with alternative splicing of exon 1B) or for rs10954213 (which directly disrupts the polyadenylation signal of *IRF5*). Because investigators in the European study only reported the data for rs2004640 (9) and because the haplotype structure of the *IRF5* region differs considerably between Caucasians and Japanese (7), it remains unclear at this point whether rs2280714 also shows the strongest association in Caucasians. Interestingly, a previous study demonstrated that the association of rs2280714 remained significant after conditioning by rs10954213, suggesting that the effect of rs2280714 cannot be fully explained by LD with rs10954213 (5).

SNP rs2280714 is located only 223 bp downstream of the 3′-UTR of *TNPO3*. TNPO3 imports multiple proteins into the nucleus, including serine/arginine-rich protein (one of the substrates of topo I), which regulates the splicing of mRNA (14). The LD block encompassing rs2280714 contains the entire *TNPO3* gene; thus, it is theoretically possible that the molecular mechanism of association with SSc may involve TNPO3. However, although the *TNPO3* mRNA level was marginally correlated with the rs2280714 genotype, the slope of the regression line and the r^2 value were much lower than those for the *IRF5* mRNA level. Thus, it is reasonable to assume that the molecular mechanism is largely mediated by up-regulation of *IRF5* mRNA.

The molecular mechanism by which *IRF5* confers risk of SSc requires further study. Investigators in a recent study reported that anti–topo I–containing sera induced IFNα from normal peripheral blood mononuclear cells, implicating a role of IFNα in vascular damage and lung fibrosis (15). Our observations support this hypothesis, because overexpression of *IRF-5* is supposed to promote such a pathway. The additional effect of rs2004640 in dcSSc might suggest a role of exon 1B–containing isoforms in the fibrotic phenotype. Such a hypothesis needs to be tested in the future.

Although the healthy controls were younger than the patients, this difference should not affect the results, because the risk that any of the controls would develop SSc later in life is extremely low due to the rarity of the disease. Furthermore, if such potential misclassification of future patients in the control group is taken into account, our current analysis should be interpreted as a conservative one.

In conclusion, our findings replicated the association of *IRF5* with SSc in a Japanese population, which supported a role of type I IFN in pathogenesis of SSc. Whether the causal SNP is different among populations requires further investigation.

AUTHOR CONTRIBUTIONS

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Clinical Images: Pseudorheumatoid gout
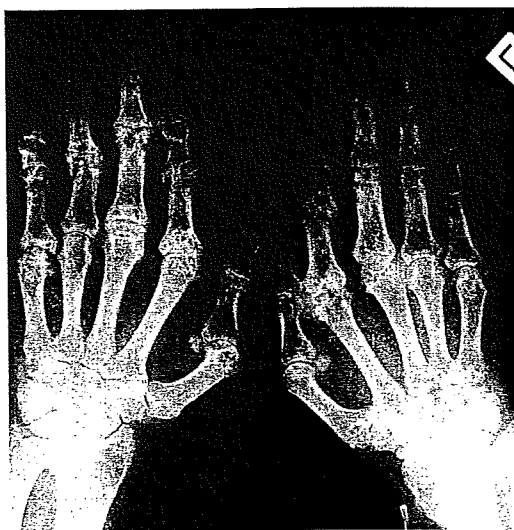
The patient, a 68-year-old man with a 30-year history of seronegative yet nodular rheumatoid arthritis, presented for reevaluation. Hand radiography (**left**) revealed soft tissue swelling and extensive joint destruction with erosions. However, because most of the erosions were "punched out" or overhanging, and because there was involvement of some of the distal interphalangeal joints and no evidence of osteopenia, the overall findings were more suggestive of gout. Aspirated material from a subcutaneous nodule (**right**) revealed colored broad plates of cholesterol crystals, which are usually nondiagnostic and which commonly complicate joint or bursa effusions of diverse etiologies. However, the presence of admixed needle-shaped negative birefringent crystals of monosodium urate monohydrate confirmed the diagnosis of gout in this patient.

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Mini Review

Role of *IRF5, STAT4* and *BLK* polymorphisms for the genetic predisposition to systemic lupus erythematosus in Japanese

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Recent large-scale association studies revealed new susceptibility genes to systemic lupus erythematosus (SLE) including *IRF5, STAT4* and *BLK*. Association of these genes have been quickly replicated by many studies in multiple populations. In this minireview, we discuss our recent studies on the association of these genes with SLE in Japanese. Although association of these genes was replicated, notable differences were observed between Caucasian and Japanese populations.

In Japanese, *IRF5* risk haplotype in the Caucasians carrying three functional polymorphisms (a single nucleotide polymorphism [SNP] at exon 1B splice site, 10 amino acid insertion/deletion, a SNP at poly A signal) is almost absent. However, another intron 1 SNP, which was not described in the Caucasians, was significantly associated with SLE in Japanese.

On the other hand, both the *STAT4* intronic SNPs and *C8orf13-BLK* intergenic SNPs associated in Caucasians were similarly associated with SLE in Japanese. Moreover, because of higher population frequencies of the risk genotypes and higher odds ratios, the contribution of these genes appeared to be greater in the Japanese than in the Caucasians.

Although association of these genes with SLE is established, the molecular mechanisms of the association remain largely unknown. In addition, further studies are required to develop applications of the genetics information for clinical use. Genetics finally began to reveal new and reliable clues to gain insight into the pathogenesis of highly complicated disorders such as SLE.

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Introduction

Epidemiological data strongly implicate significant contribution of genetic background in the development of systemic lupus erythematosus (SLE). *HLA-DRB1, C4, FCGR2A, 2B, 3A* and *3B* polymorphisms have been repeatedly associated with susceptibility to SLE[1-3]. However, a number of other susceptibility
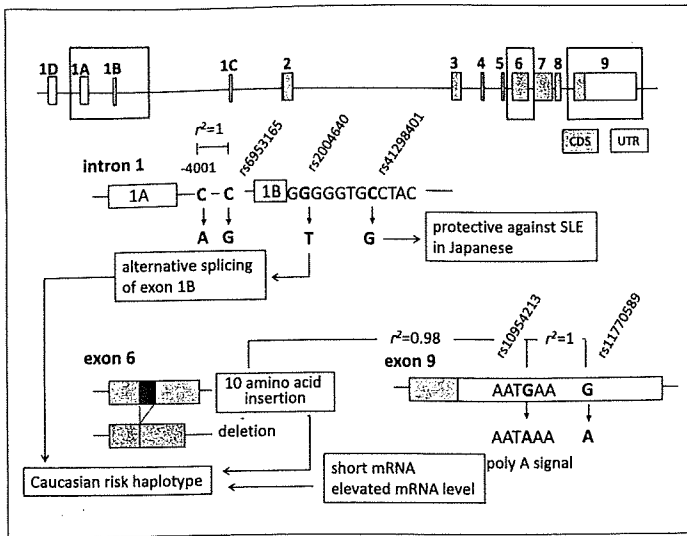
Fig.1   Difference in the *IRF5* polymorphisms associated with SLE between Caucasians and Japanese

The linkage disequilibrium parameter r^2 in the Japanese is shown. Only one of exon1 1A, 1B, 1C or 1D is used for each *IRF5* mRNA. Exon 1B is alternatively spliced only when rs2004640T allele is present. The SNP rs10954213 at exon 9 alters poly A signal, and rs10954213A allele is associated with short mRNA and elevated mRNA level. The risk haplotype for SLE in Caucasians contain rs2004640T, exon 6 insertion and rs10954213A[13-15]. However, because of strong linkage disequilibrium between exon 6 insertion and rs10954213G in the Japanese population (r^2=0.98), this haplotype is almost absent in the Japanese. Instead, rs41298401G is significantly associated with protection against SLE in Japanese[9]. CDS: coding sequence, UTR: untranslated region.

genes remain to be discovered.

Since 2005, a number of exciting new discoveries have been made in the field of lupus genetics. A candidate pathway approach focusing on type I interferon (IFN) pathway identified association of interferon regulatory factor 5 (*IRF5*) polymorphisms with SLE[4]. Subsequently, signal transducer and activator of transcription 4 (*STAT4*), identified as a susceptibility gene to rheumatoid arthritis (RA), has been shown to be associated with SLE at the same time[5].

In parallel with these discoveries, genome-wide association studies revealed new susceptibility genes such as B lymphoid tyrosine kinase (*BLK*), integrin alpha M (*ITGAM*) and tumor necrosis factor alpha-induced protein 3 (*TNFAIP3*), in addition to confirming the strong association of *IRF5* and *STAT4*[6-8]. Importantly, association of these genes with SLE has been replicated in most of the replication studies.

In this minireview, we discuss our findings on the association of *IRF5*[9], *STAT4*[10] and *BLK*[11] polymorphisms with SLE in Japanese, focusing on the similarities and differences as compared with Caucasians.

IRF5

Type I IFN family includes at least 13 IFN α , as well as IFN β, IFN κ, IFN τ and IFN ω. Type I IFN has been heavily implicated in SLE and autoimmune diseases (reviewed in 12). Serum IFN α has been reported to be elevated in SLE. Treatment of viral hepatitis with type I IFN sometimes induces antinuclear antibodies and symptoms of autoimmune diseases. Furthermore,

mRNA expression profiling on peripheral blood cells from SLE unanimously reported overexpression of genes induced by type I IFN ("interferon signature"). These studies established crucial role of type I IFN in the pathogenesis of SLE.

These lines of evidence led the researchers to examine the contribution of type I IFN pathway-related gene polymorphisms to the genetic susceptibility to SLE. The initial study in a Swedish population identified an association of a single nucleotide polymorphism (SNP) rs2004640 in intron 1 of *IRF5* with SLE[4]. Subsequently, this association was replicated in multiple large-scale studies in Caucasians[13-16], African-Americans[17] and Asians[18].

IRF5 is a transcription factor constitutively expressed in lymphocytes and dendritic cells (DCs), but is induced in other cells by viral infection and IFN α [19]. Upon stimulation of TLR7/8/9 or viral infection, IRF5 becomes activated, dimerizes and translocates to nucleus, where it induces proinflammatory cytokines such as TNF α IL-12 and IL-6, as well as type I IFN[12]. *IRF5* gene is encoded on chromosome 7q32.

During the course of association studies, it was revealed that *IRF5* contains at least 3 polymorphisms of direct functional significance (Fig.1). *IRF5* has more than 10 alternative isoforms, each contains one of exon 1A, 1B, 1C or 1D. The intron 1 SNP rs2004640 creates a splice donor site, and exon 1B is used only in the mRNA transcribed from the rs2004640T allele[13]. Secondly, there is an insertion/deletion (indel) polymorphism in exon 6 that results in indel of 10 amino acids in the PEST domain[14-16]. The functional significance of this indel is not yet clear. Thirdly, a SNP in 3'-untranslated region (3'UTR), rs10954213A>G, abol-

ishes the poly A signal. The G allele leads to the usage of the second poly A signal located 648 bp downstream, resulting in longer mRNA and reduced mRNA level[14-16]. The risk haplotype in Caucasians contains rs2004640T (exon 1B usage), exon 6 insertion and rs10954213A (elevated mRNA level)[14-16].

To examine the role of *IRF5* polymorphisms in Japanese SLE, we carried out an association study on 277 patients and 201 controls[9]. The intron 1 rs2004640T showed a tendency of association also in Japanese, but both the odds ratio (OR 1.24, 95% confidence interval [CI] 0.94-1.64, p=0,124) and the allele frequency in the controls (0.301) were smaller in Japanese as compared with the Caucasians (OR 1.47, allele frequency 0.51)[13]. On the other hand, OR and allele frequency in Japanese were very similar to those in a Korean population[18].

In contrast, 3 SNPs closely located to rs2004640 were identified, which had not previously been described in Caucasians (Fig.1). Interestingly, those SNPs showed stronger association with SLE than rs2004640 in Japanese. Specifically, rs41298401, located 6 bp downstream to rs2004640, showed the most significant protective association with Japanese SLE (G allele frequency, OR 0.65, 95%CI 0.46-0.93, p=0.017). Another SNP, rs6953165, demonstrated positive association with SLE (allele frequency, OR 1.76, 95%CI 1.04-2.97, p=0.034).

In contrast, exon 6 indel and poly A site SNP (rs10954213) did not show association with SLE in Japanese.

Haplotype analysis revealed considerable difference in the haplotype structure in Japanese and Caucasian populations. In Japanese, exon 6 insertion was in almost complete linkage disequilibrium (LD) with rs10954213G allele associated with low expression of IRF5 mRNA (r^2=0.98); thus, the risk haplotype containing both the exon 6 insertion and rs10954213A allele was almost absent in Japanese. Such a difference in the haplotype structure is likely to be associated with the difference in the associated SNP between populations. Nevertheless, the association between *IRF5* and SLE in both populations despite such a difference supports the crucial role of IRF5 in the development of SLE.

To elucidate the functional significance of intron 1 SNPs, we employed mRNA expression profile data of the lymphoblastoid B cell lines established from donors of the International HapMap project, deposited in GENEVAR database (Wellcome Trust Sanger Institute, http://www.sanger.ac.uk/humgen/genevar/). By utilizing these data in combination with the HapMap genotype data, one can statistically analyze association between any genotype and mRNA level of any gene as long as it is expressed in B cells. Actually, rs10954213 genotype was shown to be strongly

correlated with mRNA level of IRF5[9], as was experimentally demonstrated[14,15]. In addition, this genotype was also significantly correlated with a number of genes induced by IRF5[9], suggesting that this method can detect not only direct *cis*-acting effects, but also indirect *trans*-acting effects of SNPs on gene expression.

Using this approach, we examined association of intron 1 SNP genotypes with 31 genes induced by IRF5. The risk genotype of rs41298401was positively associated with expression level of *IFNA8, STAT3, STAT5B* and *TMPO* (thymopoietin), and negatively with *IFNA10*. Although the significance of these genes in relation to the molecular mechanisms of SLE is at this point unclear, these findings suggested that rs41298401 or other polymorphism which is in linkage disequilibrium with this SNP has an effect on the expression levels of some of the IRF5 inducible genes[9].

Recent studies in other populations have reported genetic effects of other *IRF5* polymorphisms, such as rs729302 in the 5'-flanking region[20], CGGGG indel[21] and rs3807306 in intron 1[22], and rs10488631 in the 3'-flanking region[20,21]. Furthermore, association of *IRF5* with other autoimmune or inflammatory diseases such as RA[23], Sjögren syndrome[24] and inflammatory bowel diseases[25] has been reported. Thus, *IRF5* is undoubtedly an established susceptibility gene to SLE and other inflammatory diseases, but the causative SNP may vary among populations, and the molecular mechanism of association requires further study.

STAT4

STAT4, located at 2q32.2-q32.3, has recently been identified as a shared susceptibility gene to RA and SLE in Caucasians[5]. Association with SLE was confirmed by two genome-wide association studies in Caucasians[6,7], and studies focused on the *STAT4* in Caucasians[26-28], Colombians[29] and Japanese[30].

STAT4 is a transcription factor expressed in lymphocytes, macrophages, and dendritic cells. STAT4 is essential for IL-12 signaling and induces IFNγ production and Th1 differentiation[31]. STAT4 is also activated by type I IFNs[32]. Furthermore, a recent study suggested the role of STAT4 in IL-23-induced IL-17 production[33]. Based on these findings, *STAT4* is considered an attractive candidate susceptibility gene to diseases rheumatic or autoimmune diseases.

STAT1 gene is located adjacently to *STAT4* at 2q32.2. STAT1 is activated by type I IFNs and IFNγ[34]. Moreover, STAT1 has been reported to be upregulated in peripheral blood mononuclear cells from SLE patients and in kidneys of lupus mice with nephritis[35,36]. These observations suggest that *STAT1* is also a strong candidate susceptibility gene to SLE.

Table 1　Population attributable risk percent (PAR%) of SLE susceptibility alleles

gene	allele	population	model	population frequency of the risk genotype	OR of the risk genotype	PAR%	reference
STAT4	rs7574865T	Japanese	dominant	0.565	2.19	40.2%	10
C8orf13-BLK	rs13277113A	Japanese	recessive	0.432	2.27	35.4%	11
IRF5	rs41298401C	Japanese	recessive	0.652	1.55	26.4%	9
HLA-DRB1	DRB1*1501	Japanese	dominant	0.124	2.97	19.6%	39
FCGR2B	rs1050501C	Japanese	recessive	0.053	2.19	5.9%	40
TNFRSF1B(TNFR2)	rs60195947G	Japanese	dominant	0.188	2.53	22.4%	41
TNFSF13(APRIL)	rs11552708G	Japanese	dominant	0.803	2.01	44.7%	42
STAT4	rs7574865T	Caucasians	dominant	0.412	1.59	19.5%	6
C8orf13-BLK	rs13277113A	Caucasians	dominant	0.406	1.48	16.2%	10

PAR% were calculated based on the model (dominant or recessive) which gave a smaller P value by χ^2 test using 2 x 2 contingency tables for each allele.

In order to comprehensively examine the role of *STAT1-STAT4* region for SLE, we selected 52 tag SNPs encompassing this region, and carried out an association study in Japanese[10]. Among the tag SNPs, rs10168266 in intron 5 as well as rs11889341 and rs7574865 in intron 3 were most significantly associated with SLE. In contrast, significant association was not detected for SNPs in the *STAT1* region.

The rs7574865T allele, previously shown to be associated with SLE in Caucasians, was significantly increased in Japanese SLE (0.463) compared with controls (0.335, $p=4.9 \times 10^{-6}$, OR 1.71, 95%CI 1.36-2.15). The association was compatible with the dominant model, under which the OR was 2.19 (T/T + G/T versus G/G). The SNPs rs11889341 and rs10168266 were in LD with rs7574865 and were also significantly associated with SLE. Logistic regression analysis failed to identify a single causative SNP of the three due to the strong LD.

Association of these SNPs was more strongly observed in SLE patients with nephritis or with anti-dsDNA antibodies both in the Caucasians[26,37] and in the Japanese[10].

The functional significance of these intronic SNPs remains unclear, but recent studies indicated that the risk genotype is associated with elevated mRNA level of STAT4[28,37], but not with splicing isoform[28].

C8orf13-BLK region

C8orf13-BLK region at 8p23.1 is a recently identified susceptibility region for SLE by two genome-wide association studies in Caucasian populations[6,7]. *BLK* encodes a B lymphoid specific tyrosine kinase of Src family[38], whose function remains unclear. *C8orf13* is a ubiquitously expressed gene, the function

of which also remains unknown. The SLE-associated SNP, rs13277113, is located at the intergenic region between these genes, and the risk allele has been shown to be associated with low mRNA levels of *BLK* and high mRNA levels of *C8orf13*[6].

To test whether this region is also associated with SLE in a Japanese population, we carried out an association study for 14 tag SNPs in this region. Eleven of the 14 SNPs exhibited evidence for association, among which rs13277113 showed the strongest association (allele frequency $p=4.75 \times 10^{-7}$, OR 2.44, 95%CI 1.43-4.16). Most of the effects of other SNPs could be accounted for by LD with rs13277113. Thus, *C8orf13-BLK* region appears to contain shared susceptibility factor at least between Caucasians and Japanese[11].

Contribution of each risk allele in different populations

Contribution of each susceptibility gene to diseases substantially varies among populations, at least partly because of differences in the allele frequencies, haplotype structure and possibly in the interacting genetic and environmental factors. To compare the impact of each risk allele in each population, we estimated population attributable risk percent (PAR%) of *IRF5*, *STAT4*, *BLK* as well as other previously established susceptibility genes to SLE in Japanese, such as *HLA-DRB1*1501*[39], *FCGR2B*[3,40], *TNFRSF1B(TNFR2)*[41] and *TNFSF13(APRIL)*[42]. PAR% is "used to estimate the excess rate of disease in the total study population of exposed and nonexposed that is attributable to the exposure"[43], which, in this case, is the risk genotype. In a case-control study of a rare disease like SLE, PAR% can be approximated using the OR and the risk genotype frequency in the healthy

controls. The sum of PAR% for all risk alleles exceeds 100%, because each individual carries multiple risk alleles in a multi-factorial disease like SLE.

As shown in Table 1, PAR% of *STAT4* and *BLK* were considerably greater in the Japanese compared with the Caucasians. This is because both the OR and population frequency of the risk genotype are greater in Japanese[10,11]. Furthermore, PAR% of *STAT4* and *BLK* are also greater than those of most of the other susceptibility genes in the Japanese.

Because PAR% may be affected by the difference in the method of ascertainment of each study, comparison of PAR% among different studies needs to be interpreted with caution. Nevertheless, these observations suggested substantial impact of these susceptibility genes on the development of SLE in Japanese.

Concluding remarks

Most of the susceptibility genes recently identified through large-scale studies have been successfully replicated in multiple populations. Furthermore, although pathways involving type I IFN, Th1/Th2 regulation and B cell receptor signaling had been heavily implicated, molecules such as IRF5, STAT4 and BLK had not been investigated in relation to the pathogenesis of SLE until the genetic association became evident. Thus, human genetics is finally beginning to disclose many new and solid paths to the understanding of the etiopathogenesis of lupus.

Our current findings emphasized that even for the susceptibility genes shared by multiple populations, difference in the associated risk allele or the degree of risk conferred by the allele should be taken into account for medical application.

The molecular mechanisms of association of these genes as well as the specific strategy how to utilize the genetics information for the diagnosis, treatment and prevention of lupus will require years of intensive research. At this point, however, it is fair to say that the lupus genetics research is on the right track.

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A case of polyarteritis nodosa with periurethral aseptic abscesses and testicular lesions

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ABSTRACT
We describe a 54-year-old man presenting with cutaneous ulcerations, livedo reticularis, numbness of the legs, and skin histological findings compatible with the diagnosis of polyarteritis nodosa (PAN). Initial treatment with 50 mg/day of prednisolone (PSL) was effective. However, the symptoms and signs recurred, and the patient developed multiple periurethral aseptic abscesses, urethra-cutaneous fistula, and testicular lesions after tapering of PSL therapy. The condition improved with PSL and cyclophosphamide administration. Since penile and testicular vasculitis could be associated with PAN, although rarely, we should carefully distinguish such an involvement from infection and malignancy.

Introduction

Polyarteritis nodosa (PAN) is a necrotizing inflammation of medium-sized or small arteries, without glomerulonephritis or vasculitis in the arterioles, capillaries and venules (1). Although the testis is found to be frequently involved at autopsy, clinical presentation suggestive of testicular involvement is uncommon (2). We report a case of PAN with periurethral aseptic abscesses, urethra-cutaneous fistula, and masses in the testis.

Case report

A 54-year-old man was referred to our hospital in May 2003 with a 2-year history of cutaneous ulcerations, *livedo reticularis* and numbness of the legs. Skin biopsy from the planta pedis revealed necrotizing vasculitis (leukocyte infiltration and fibrin deposition in the arterial wall) of medium-sized arteries in the lower part of the dermis. He was diagnosed as having PAN, since presence of *livedo reticularis*, mononeuritis multiplex and histological findings of the skin fulfilled the diagnosis criteria of PAN according to the American College of Rheumatology (3). The initial treatment with 50 mg of oral prednisolone (PSL) daily was effective. However, since leg ulceration recurred after tapering of PSL to 10 mg/day in March 2004, it was considered that the disease had relapsed, and then low-dose of oral

methotrexate (5 mg/week) was added to the 10 mg/day PSL. Three months later, the patient noticed penile swelling. Antibiotic treatment (levofloxacin 300mg/day) was not effective. Penile centesis revealed aseptic abscess, with negative tests for bacterial and tuberculosis culture and DNA-polymerase chain reaction for tuberculosis. Magnetic resonance imaging (MRI) of the pelvis showed multiple high-intensity areas in the periurethra and both testes on T2-weighted images (Fig. 1). However, he did not have any symptoms with testis. In August 2004, spontaneous disintegration of the abscesses and the appearance of a urethra-cutaneous fistula were noted. The patient also reported reappearance of numbness of legs, resulting in readmission to the hospital in October 2004.

Physical examination showed *livedo reticularis* and purpura on both legs and ulcers on the right knee, left heel, and hallux. The penile fistula was noted and neurological examination showed paresthesia along the distribution of the right lateral sural nerve. Laboratory tests showed leukocytosis (12,000/ml, segmented cells 84%, lymphocytes 13%,
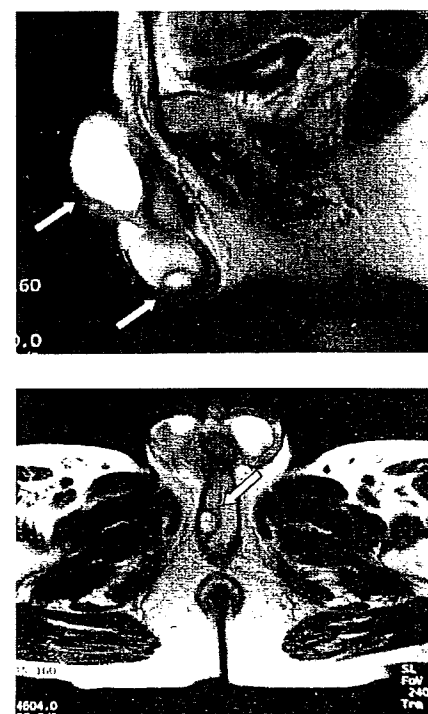

Fig. 1. T2-weighted MRI findings before admission (July 2004). Arrows indicate multiple high-intensity areas in the periurethra and testes.
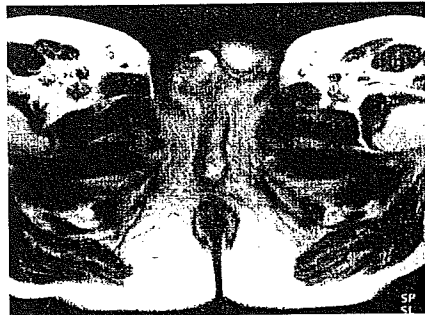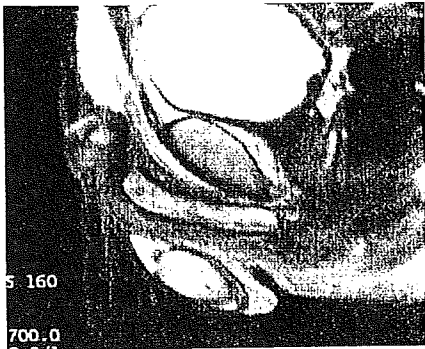
Fig. 2. T2-weighted MRI findings after treatment (January 2005). Note the disappearance of high-intensity areas in the periurethra and testes.

monocytes 2%), erythrocyte sedimentation rate of 66 mm/hr, and C-reactive protein (CRP) of 3.4 mg/dl. Tests for autoimmune antibodies, including antinuclear antibodies and anti-neutrophil cytoplasmic antibodies, and hepatitis B surface antigen were negative. Urine culture and abscess fluid were negative for microorganisms. Chest x-ray and abdominal computed tomography showed no abnormalities.

Since periurethral aseptic abscesses and masses in the testis appeared with lower limb ulcers and paresthesia, we considered all clinical features might be caused by recurrence of vasculitis. Intravenous pulse cyclophosphamide (750 mg/day) was administered. However, CRP continued to increase to 9.3 mg/dl together with deterioration of leg ulceration and severe pain. The PSL dose was increased to 50 mg/day from 10 mg/day, which resulted in overall clinical improvement. Oral cyclophosphamide (50 mg/day) was added in January 2005 and PSL was tapered. T2-weighted MRI of the pelvis in January 2005 showed disappearance of the high intensity areas in the periurethral region and testes (Fig. 2). PSL was gradually tapered to 5 mg/day without recurrence of vasculitis.

Oral cyclophosphamide was switched to azathioprine (50mg/day) in August 2006, and PSL (5mg/day) was continued. In September 2007, since ulcers of right toe skin appeared, administration of azathioprine was changed to cyclosporine (100mg/day). After that, clinical symptoms improved and the patients remained asymptomatic. The appropriate consent for this report was obtained from the patient.

Discussion

Penile involvement in PAN is very rare. To our knowledge, only two such cases have been reported (4, 5). Present case developed periurethral aseptic abscesses and urethra-cutaneous fistula, which are the first reported findings of PAN. Moreover, the involvements were improved by immunosuppressive therapy.

Testicular involvement in PAN was first reported by Monckeberg in 1905 (6). Autopsy series demonstrated testicular vasculitis in 60 to 86% of PAN patients (2), however, only 2 to 24% of patients were symptomatic (2, 3, 7). Up to now, seventeen cases with symptomatic isolated testicular vasculitis (8-22), and eleven cases who had initially symptomatic testicular vasculitis, following systemic involvement (7, 23-29), were reported. The main complaints of testicular involvement are testicular swelling, pain and testicular tumor. In twenty-two cases out of the 28 patients, testectomy or epididymectomy was performed, mostly based on suspicion of malignant testicular tumor (7-8, 10-12, 14-20, 22-23, 25-27, 29). These findings point to the difficulty in differentiating PAN testicular involvement from malignancy. Both isolated testicular vasculitis and systemic PAN show similar histological characteristics, including panarteritis, periarteritis, acute and chronic inflammation, and fibrinoid necrosis of the vessel wall, although testicular infarcts in systemic PAN were more frequent (8). In isolated testicular vasculitis, 14 patients were treated by testectomy or epididymectomy (8, 10-12, 14-22), while 3 received PSL or other immunosuppressants (13-14, 16). In patients with systemic vasculitis, 8 were treated with

PSL or other immunosuppressants (23-29), and treatment was not described in 3 (7). All patients responded well to the treatment.

In summary, PAN could be associated with vasculitis of the penile and testicular arteries. We need to carefully distinguish such an involvement from infection and malignant tumors.

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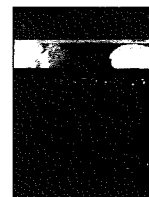
Meta-analysis of association between genetic variants in *COMT* and schizophrenia: An update

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ABSTRACT

A common functional polymorphism, Val108/158Met (rs4680), and haplotypes rs737865–rs4680–rs165599 in the Catechol-O-methyltransferase gene (*COMT*) have been extensively examined for association to schizophrenia; however, results of replication studies have been inconsistent. The aim of this study was to comprehensively evaluate the genetic risk of *COMT* for schizophrenia.

First, we performed a mutation scan to detect the existence of potent functional variants in the 5′-flanking and exon regions. Second, we conducted a gene-based case–control study between tagging single nucleotide polymorphisms (SNPs) in *COMT* [19 SNPs including six possible functional SNPs (rs2075507, rs737865, rs4680, rs165599, rs165849)] and schizophrenia in large Japanese samples (schizophrenics 1118, controls 1100). Lastly, we carried out a meta-analysis of 5 functional SNPs and haplotypes (rs737865–rs4680–rs165599).

No novel functional variant was detected in the mutation scan. There is no association between these tagging SNPs in *COMT* and Japanese schizophrenia. In this updated meta-analysis, no evidence was found for an association between Val108/158Met polymorphisms, rs6267, rs165599, and haplotypes (rs7378655–rs4680–rs165599) and schizophrenia, although rs2075507 and rs737865 showed trends for significance in allele-wise analyses ($P = 0.039$ in a multiplicative model, $P = 0.025$ in a recessive model for rs2075507, $P = 0.018$ in a dominant model for rs737865, uncorrected). This significance did not remain, however, after correcting the P-values using a false discovery rate controlling procedure.

Our results suggest that the *COMT* is unlikely to contribute to susceptibility to schizophrenia.

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1. Introduction

The gene encoding Catechol-O-methyltransferase (*COMT*) is considered to be a likely candidate gene for schizophrenia

owing to 1) the role of the enzyme in dopamine metabolism and to 2) its chromosomal location, 22q11, which has been implicated in schizophrenia by several linkage studies (Owen et al., 2005), as well as by its deletion in Velo-cardio-facial syndrome, in which patients frequently develop psychotic disorders including schizophrenia (Murphy et al., 1999).

Most *COMT* genetic association studies have focused on a particular single nucleotide polymorphism (SNP) that results in a change from Valine to Methionine at codon 158/108

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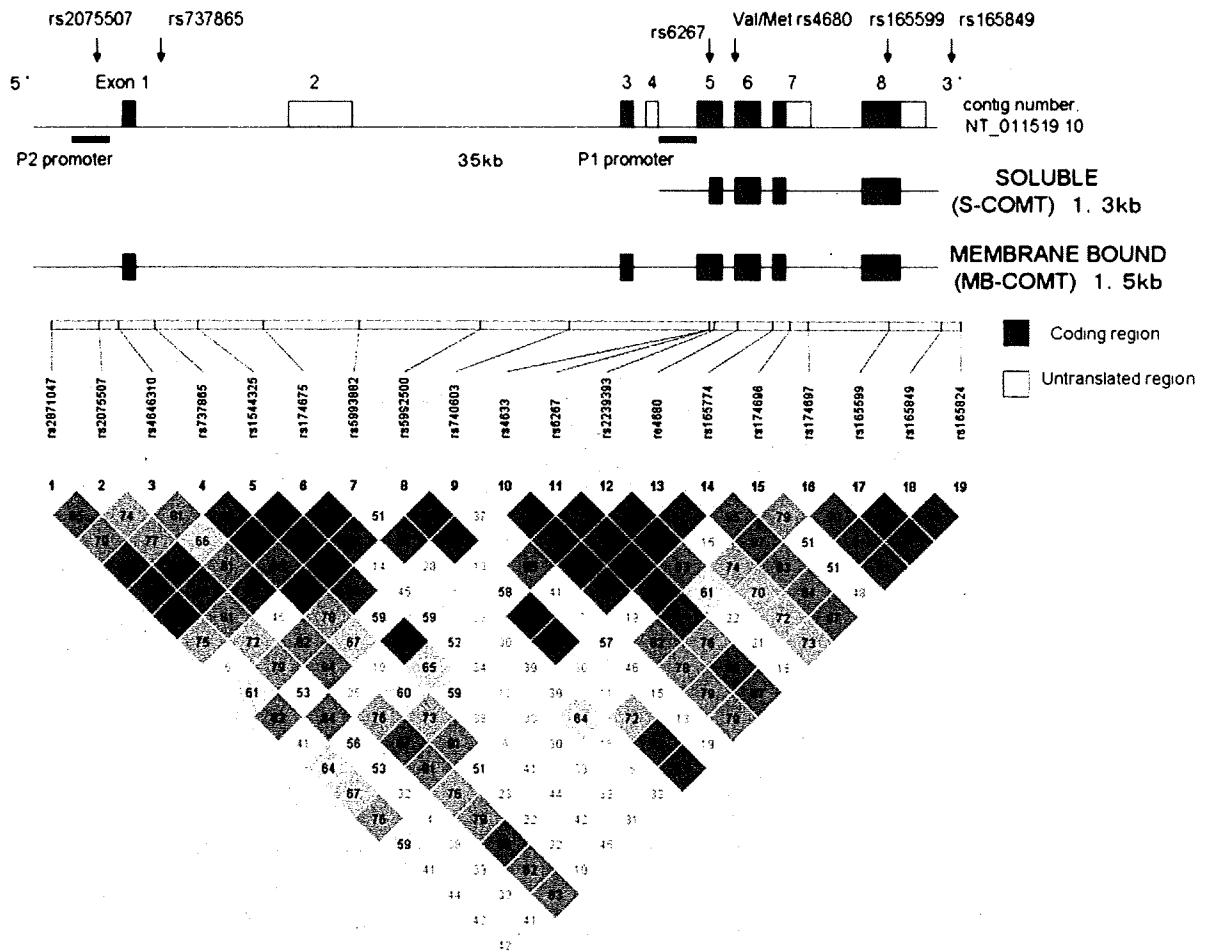
Chromosome 22q11

Fig. 1. COMT structure and positions of 'tag SNP' in control sample. LD structure (D') was evaluated with the use of Haploview software.

(rs4680) in COMT (Lachman et al., 1996). This is because the Val158/108Met polymorphism has possible functional relevance; the Val allele leads to efficient degradation of dopamine and lower than normal dopamine levels in the brain, since it has higher enzymatic activity than the Met variant (Chen et al., 2004). In addition, recent studies have shown that this possible functional polymorphism (Val/Met) affects executive cognition in the prefrontal cortex (PFC) during working memory (Egan et al., 2001). The above findings suggest that the Val allele may contribute to increased prefrontal dopamine catabolism and impaired PFC function.

A large number of case–control association studies between the Val/Met polymorphism in COMT and schizophrenia have been conducted, but the results have been inconsistent; some studies showed a significant association between the Val allele and schizophrenia (Egan et al., 2001; Handoko et al., 2005; Kremer et al., 2003; Li et al., 2000; Shifman et al., 2002; Wonodi et al., 2003), whereas others indicated that the Met allele was associated with schizophrenia (Ohmori et al., 1998; Sazci et al., 2004). The majority, however, found no association (Arinami et al., 2001; Chen et al., 1999; Daniels et al., 1996; Fan et al., 2005; Galderisi et al., 2005; Gallinat et al., 2003; Goghari and

Sponheim, 2008; Golimbet et al., 2006; Han et al., 2004, 2006; Herken et al., 2003; Illi et al., 2003; Inada et al., 2003; Iwata et al., 2003; Joo et al., 2005; Joober et al., 2002; Karayiorgou et al., 1998; Kotler et al., 1999; Krabbendam et al., 2006; Liou et al., 2001; Martorell et al., 2008; Muntjewerff et al., 2008; Nicodemus et al., 2007; Numata et al., 2006; Nunokawa et al., 2007; Ohnishi et al., 2006; Park et al., 2002; Poyurovsky et al., 2005; Rujescu et al., 2003; Sanders et al., 2008; Semwal et al., 2002; Strous et al., 1997; Szoke et al., 2006; Thaker et al., 2004; Williams et al., 2005; Yu et al., 2007). In addition, three meta-analyses of this SNP have been reported, but their results were also inconsistent. The first, by Glatt et al. (2003), reported that in case–control studies, especially family-based studies, the Val allele was associated with schizophrenia in the European population. The other two analyses did not provide evidence for a significant association between the Val allele and schizophrenia in either European or Asian populations (Fan et al., 2005; Munafo et al., 2005).

One possible cause of this inconsistency is the existence of an actual causal variant in linkage disequilibrium (LD) with Val/Met polymorphism. Some studies support this, showing a more significant association in haplotypes or other functional

SNPs than from the Val/Met polymorphism alone (Funke et al., 2005; Lee et al., 2005; Sanders et al., 2005; Shifman et al., 2002). For example, (Shifman et al. (2002) reported that haplotypes constructed by three SNPs (Val/Met, rs737865, rs165599) showed the strongest association with schizophrenia in Ashkenazi Jews ($P = 9.5 \times 10^{-8}$).

COMT encodes two transcripts from two promoters in humans (membrane bound; MB-COMT of 1.5 kb from P2/soluble; S-COMT of 1.3 kb from P1: Fig. 1). Funke et al. and Lee et al. also showed that other functional SNPs, rs2075507 (in P2 promoter region of MB-COMT) and rs6267 (change from Alanine to Serine at codon 22/72 in the MB-COMT/S-COMT), were associated with schizophrenia in a European population (Funke et al., 2005) and in a Korean population (Lee et al., 2005).

Since differences in LD among populations may be responsible for the inconsistency of these results, it is important in association studies to select informative genetic variants (e.g. tagging SNPs) that adequately reflect the LD background in the targeted population. Therefore, re-sequencing for mutation screening and LD-based (or gene-based) association studies (Neale and Sham, 2004) is essential to examine the association between COMT and schizophrenia.

In this study, we carried out a systematic mutation scan in the 5' region and all exon regions and a gene-based case-control study using 19 tagging SNPs in a Japanese sample. We also included an updated meta-analysis for not only Val/Met polymorphism but also other functional SNPs and haplotypes that have been intensively investigated in other studies (rs207055, rs737655, rs6267, rs165599, and rs737865-rs4680-rs165599).

2. Materials and methods

2.1. Mutation scan and case-control study in the Japanese population

2.1.1. Subjects

The subjects in the association analysis were 1118 schizophrenia patients (628 males and 490 females; mean age ± standard deviation; 45.4 ± 15.5 years) and 1100 healthy controls (504 males and 596 females; 38.1 ± 15.2 years). The subjects for the mutation search were 96 patients with schizophrenia. These subjects were also included in the association analysis. All subjects were unrelated to each other and
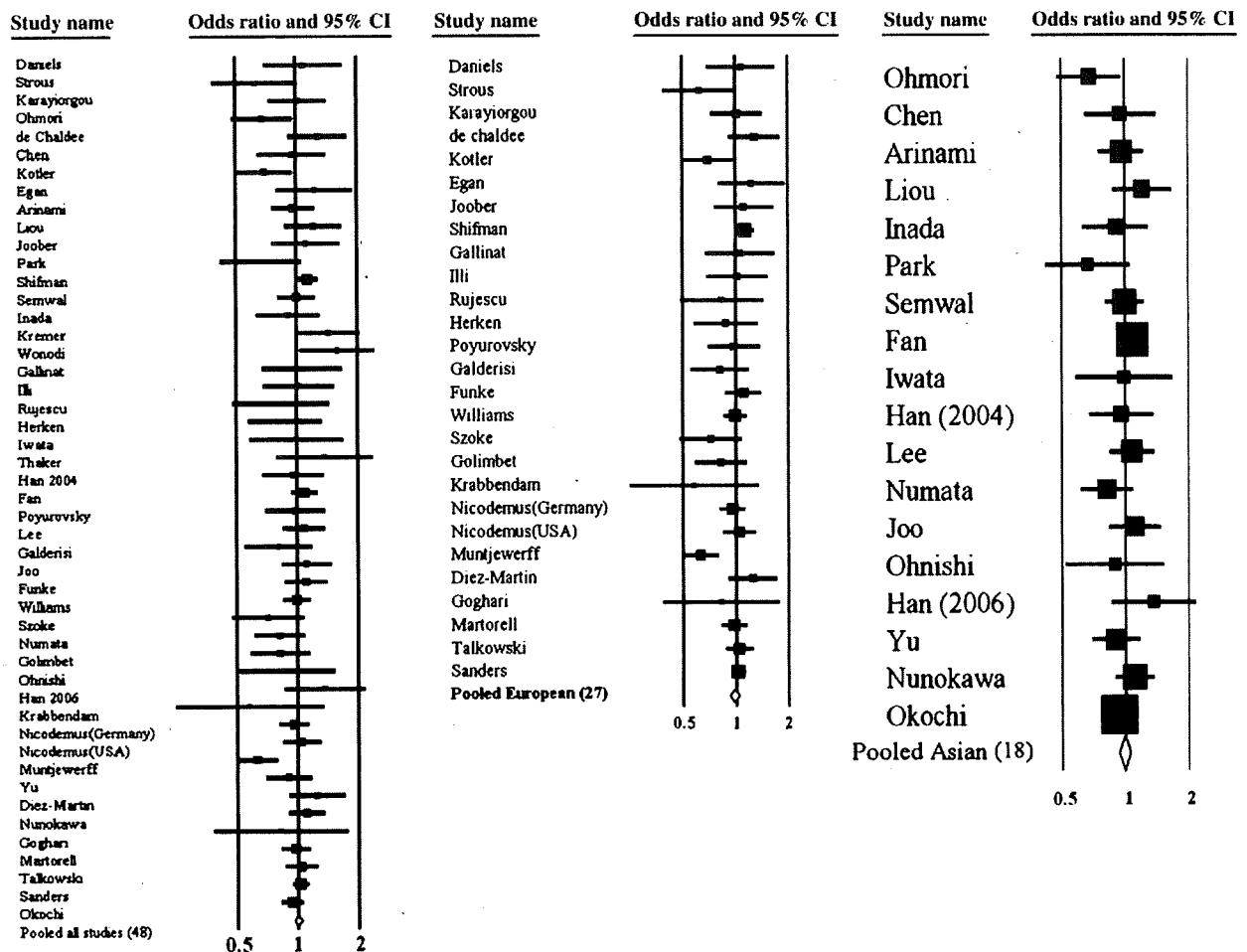
Fig. 2. Forest plots of OR with 95% CI for the Val/Met polymorphism. Results of all pooled studies and subgroup analyses are shown.