

# Studies on the association of Fc $\gamma$ receptor IIA, IIB, IIIA and IIIB polymorphisms with rheumatoid arthritis in the Japanese: evidence for a genetic interaction between HLA-DRB1 and FCGR3A

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We recently detected a new single nucleotide polymorphism of Fc $\gamma$ RIIB gene, which alters an amino acid within the transmembrane domain from Ile to Thr (I232T), and its association with SLE in the Japanese. This study was performed to examine whether FCGR2B-I232T was associated with susceptibility to rheumatoid arthritis in the Japanese. At the same time, FCGR2A, 3A and 3B polymorphisms were also examined. Genotyping of FCGR2B-I232T, FCGR2A-H131R, FCGR3A-F176V and FCGR3B-NA1/2 polymorphisms were performed using genomic DNA. Association with RA was analyzed in 382 Japanese patients with RA and 303 healthy individuals using a case-control approach. In addition, the same groups of patients and controls were genotyped for HLA-DRB1 to examine possible interaction with FCGR genes. Significantly different distribution of genotype, allele carrier and allele frequencies was not observed between patients with RA and healthy individuals in any of the four polymorphisms. When the subjects were stratified according to the carriage of HLA-DRB1 shared epitope (SE), significant increase of FCGR3A-176F/F genotype was observed in SE positive patients compared with SE positive healthy individuals ( $P = 0.009$ ,  $P_{\text{corr}} = 0.07$ ). In conclusion, FCGR3A-176F/F genotype was considered to confer risk through genetic interaction with HLA-DRB1 SE.

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**Keywords:** Fc $\gamma$  receptor; FCGR3A polymorphism; rheumatoid arthritis; HLA-DRB1 shared epitope

## Introduction

Fc $\gamma$  receptors (Fc $\gamma$ Rs) are members of the immunoglobulin superfamily that bind to the Fc portion of IgG. Human low-affinity Fc $\gamma$ Rs consist of three Fc $\gamma$ RII (IIA, IIB, IIC) and two Fc $\gamma$ RIII (IIIA, IIIB), which vary in their cellular distribution and affinity for different IgG isotypes. Fc $\gamma$ Rs are expressed in immune effector cells and are thought to play an important role in the pathogenesis of autoimmune diseases.<sup>1</sup>

Among the five Fc $\gamma$ Rs genes (FCGR the designation using Roman numerals indicate the polypeptide product, while the designation using Arabic numerals refers to the corresponding locus), FCGR2A-H131R, FCGR3A-F176V (often designated as F158V by counting from the N-terminal amino acid of the mature protein, excluding the signal peptide) and FCGR3B-NA1/2 polymorphisms had previously been known.<sup>1</sup> Association of FCGR polymorphisms has been extensively studied in a variety of

autoimmune and/or inflammatory diseases. Recently, we identified a single nucleotide polymorphism (SNP) in Fc $\gamma$ RIIB gene coding for a nonsynonymous substitution within the transmembrane domain, Ile232Thr (I232T), and demonstrated that FCGR2B-232T/T genotype was significantly increased in Japanese patients with systemic lupus erythematosus (SLE).<sup>2</sup>

Rheumatoid arthritis (RA) is a systemic autoimmune disease, characterized by chronic inflammation of the synovial as well as extra-articular tissues. Currently, the only established susceptibility gene to RA is HLA class II. The association of certain HLA-DRB1 alleles encoding the 'shared epitope' (SE) with susceptibility to RA is widely accepted.<sup>3</sup> In Japanese, unlike in Caucasians, the most common allele of SE is DRB1\*0405; nevertheless, significant association with SE has been confirmed.<sup>4</sup>

Functional importance of Fc $\gamma$ Rs in the pathogenesis of RA has been shown by animal studies. DBA/1 mice lacking Fc $\gamma$  chain, and thus lacking Fc $\gamma$ RI and Fc $\gamma$ RIII signaling, were protected from collagen-induced arthritis (CIA), while DBA/1 mice lacking Fc $\gamma$ RIIB developed arthritis.<sup>5</sup> 129/SvJ (H-2<sup>b</sup>) and C57BL/6 (H-2<sup>b</sup>) hybrid background mice, usually resistant to CIA, became susceptible to CIA and developed cartilage destruction when Fc $\gamma$ RIIB gene deficiency was introduced.<sup>6</sup> In humans, only a limited number of studies have been reported on the association of FCGR polymorphisms with RA. In Caucasians living in southern Spain,<sup>7</sup> in the United Kingdom (UK),<sup>8</sup> and in North Indians/

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Pakistanis,<sup>8</sup> *FCGR3A-176F/V* polymorphism was reported to be associated with RA. However, the allele showing higher genetic risk for RA was opposite in these two studies. In the previous study, we reported lack of significant association of *FCGR2A*, *3A* and *3B* polymorphisms with RA in 115 Japanese patients and 217 healthy individuals.<sup>9</sup> However, *FCGR2B* polymorphism, which was considered to be most relevant to pathogenesis, has not been examined for association with RA.

The purpose of this study was to investigate the association of *FCGR2B-I232T* with RA. At the same time, we evaluated the association of *FCGR2A*, *3A* and *3B* in a larger sample size. In addition, possible genetic interaction between *FCGRs* and *HLA-DRB1* was examined.

## Results

### Genotyping of *FCGR2B*, *2A*, *3A* and *3B* gene polymorphisms

Table 1 shows the frequencies of *FCGR2B-I232T*, *FCGR2A-H131R*, *FCGR3A-F176V* and *FCGR3B-NA1/2* genotypes in 382 patients and 303 healthy individuals. Significant difference in genotype frequencies was not observed between patients and healthy individuals in all

*FCGR* gene polymorphisms. Accordingly, allele carrier frequencies and allele frequencies did not show any difference between patients and healthy individuals.

Genotype relative risk estimation by the method of Lathrop adjusts control data for Hardy–Weinberg equilibrium and thus reduces variance.<sup>10</sup> This type of analysis did not reveal association of any of the *FCGR* genes with RA either. Since a tendency of increase in the genotype frequency of *FCGR3A-176F/F* was observed, association of *FCGR3A* was also analyzed using Armitage’s test for trend.<sup>11</sup> This analysis resulted in *P*-value of 0.15, but the difference still did not reach statistical significance.

Analyses on the association of *FCGR2B*, *2A*, *3A* and *3B* polymorphisms with disease phenotypes in patients with RA, such as age of onset under 40 and presence of rheumatoid factor (RF), did not reveal significant association either (data not shown).

We next examined whether any of the haplotypes formed by four *FCGR* alleles is more frequently found in RA compared with controls. Since the genotypes of the family of the subjects were not available, haplotype frequencies were estimated using EH program.<sup>12</sup> As shown in Table 2, the estimated haplotype frequencies were quite similar in RA and healthy individuals. Haplotypes formed by *FCGR2A-131H*, *3A-176F*, *3B-NA1*

**Table 1** *FCGR2B*, *FCGR2A*, *FCGR3A* and *FCGR3B* polymorphisms in Japanese patients with RA and healthy individuals

		RA (n=382)		Healthy individuals (n=303)		P-value
<i>Genotype frequency<sup>a</sup></i>						
<i>FCGR2B</i>	<i>232I/I</i>	224	(58.6)	183	(60.4)	NS
	<i>232I/T</i>	135	(35.4)	104	(34.3)	
	<i>232T/T</i>	23	(6.0)	16	(5.3)	
<i>FCGR2A</i>	<i>131R/R</i>	20	(5.2)	11	(3.6)	NS
	<i>131R/H</i>	111	(29.1)	95	(31.4)	
	<i>131H/H</i>	251	(65.7)	197	(65.0)	
<i>FCGR3A</i>	<i>176F/F</i>	205	(53.7)	145	(47.8)	NS
	<i>176V/F</i>	149	(39.0)	132	(43.6)	
	<i>176V/V</i>	28	(7.3)	26	(8.6)	
<i>FCGR3B</i>	<i>NA2/2</i>	43	(11.2)	42	(13.9)	NS
	<i>NA1/2</i>	197	(51.6)	145	(47.8)	
	<i>NA1/1</i>	142	(37.2)	116	(38.3)	
<i>Allele carrier frequency<sup>b</sup></i>						
<i>FCGR2B</i>	<i>I</i> present	359		287	(94.7)	NS
	<i>T</i> present	158	(41.4)	120	(39.6)	NS
<i>FCGR2A</i>	<i>R</i> present	131	(34.3)	106	(35.0)	NS
	<i>H</i> present	362	(94.8)	292	(96.4)	NS
<i>FCGR3A</i>	<i>F</i> present	354	(92.7)	277	(91.4)	NS
	<i>V</i> present	177	(46.3)	158	(52.1)	NS
<i>FCGR3B</i>	<i>NA2</i> present	240	(62.8)	187	(61.7)	NS
	<i>NA1</i> present	339	(88.7)	261	(86.1)	NS
<i>Allele frequency<sup>b</sup></i>						
<i>FCGR2B</i>	<i>I</i> allele	583	(76.3)	470	(77.6)	NS
	<i>T</i> allele	181	(23.7)	136	(22.4)	
<i>FCGR2A</i>	<i>R</i> allele	151	(19.8)	117	(19.3)	NS
	<i>H</i> allele	613	(80.2)	489	(80.7)	
<i>FCGR3A</i>	<i>F</i> allele	559	(73.2)	422	(69.6)	NS
	<i>V</i> allele	205	(26.8)	184	(30.4)	
<i>FCGR3B</i>	<i>NA2</i> allele	283	(37.0)	229	(37.8)	NS
	<i>NA1</i> allele	481	(63.0)	377	(62.2)	

Numbers in parentheses indicate the percentage.

<sup>a</sup>*P*-values were calculated by  $\chi^2$  test  $3 \times 2$  contingency table (df=2).

<sup>b</sup>*P*-values were calculated by  $\chi^2$  test  $2 \times 2$  contingency table (df=1).

**Table 2** Estimated haplotype frequencies formed by four *FCGR*s alleles in the Japanese patients with RA and healthy individuals

Haplotypes <sup>a</sup>				Healthy individuals (%)	RA (%)
2A	3A	3B	2B		
131H	176F	NA1	232I	34.2	36.9
131H	176V	NA1	232I	17.3	14.5
131H	176F	NA2	232T	15.2	14.6
131R	176F	NA2	232I	7.0	6.8
131H	176F	NA2	232I	5.3	5.7
131R	176F	NA1	232I	5.2	5.2
131R	176V	NA1	232I	4.7	4.4
131H	176V	NA2	232T	4.0	4.8
131H	176V	NA2	232I	3.8	2.6
131R	176F	NA2	232T	2.4	2.5
Others <sup>b</sup>				0.9	2.0

<sup>a</sup>Haplotype frequencies were estimated using EH program.<sup>12</sup><sup>b</sup>Haplotype frequency less than 2%.

and 2B-232I was the most common haplotype in the Japanese.

### Genetic interaction between *FCGR3A* and *HLA-DRB1* shared epitope

We next examined whether *FCGR* polymorphisms may confer susceptibility to RA in concert with the already established susceptibility gene, namely, *HLA-DRB1*. As

previously described, striking association of *HLA-DRB1* SE with susceptibility to RA was present in our subjects (Table 3).<sup>4</sup> Genotype frequency of each *FCGR* was compared between patients and controls after stratification according to the presence of SE. Significant difference in the genotype frequency was only detected in *FCGR3A-F176V* polymorphism when compared between patients with SE and healthy individuals with SE ( $P=0.03$ ,  $P_{\text{corr}}=0.24$  by  $\chi^2$  test,  $P=0.017$ ,  $P_{\text{corr}}=0.14$  by Armitage's test for trend), but not in other comparisons. This association derived from the increase of *FCGR3A-176F/F* homozygotes in patients carrying SE compared with controls carrying SE ( $\chi^2=6.8$ ,  $P=0.009$ ,  $P_{\text{corr}}=0.072$ , *F/F vs F/V and V/V* combined).

As shown in Table 4, odds ratio for the development of RA was 4.84 in individuals with SE and *FCGR3A-F/F* genotype, which was significantly higher when compared with 2.71 in those with SE, but with *FCGR3A-F/V* or *V/V* genotypes.

## Discussion

In the present study, we failed to detect significant overall association of *FCGR2A*, 2B, 3A and 3B polymorphisms with RA in the Japanese population. However, a slight trend for the increase of *FCGR3A-176F/F* was observed in RA, which reached statistical significance when this genotype was compared between

**Table 3** *FCGR* genotype frequencies in the patients and healthy individuals stratified according to *HLA-DRB1* SE

	<i>FCGR2B-I232T</i>	<i>FCGR2A-H131R</i>	<i>FCGR3A-F176V</i>	<i>FCGR3B-NA1/2</i>
SE positive <sup>a</sup>				
RA $n=270/379$ (71.2%)				
	I/I 151 (55.9)	R/R 13 (4.8)	F/F 151 (55.9) <sup>b</sup>	NA2/2 31 (11.5)
	I/T 103 (38.2)	R/H 86 (31.9)	V/F 100 (37.1)	NA1/2 137 (50.7)
	T/T 16 (5.9)	H/H 171 (63.3)	V/V 19 (7.0)	NA1/1 102 (37.8)
Healthy individuals $n=118/301$ (39.2%)				
	I/I 66 (55.9)	R/R 6 (5.1)	F/F 49 (41.5)	NA2/2 18 (15.3)
	I/T 44 (37.3)	R/H 39 (33.0)	V/F 58 (49.2)	NA1/2 61 (51.7)
	T/T 8 (6.8)	H/H 73 (61.9)	V/V 11 (9.3)	NA1/1 39 (33.0)
SE negative				
RA $n=109/379$ (28.8%)				
	I/I 70 (64.2)	R/R 7 (6.4)	F/F 53 (48.6)	NA2/2 12 (11.0)
	I/T 32 (29.4)	R/H 24 (22.0)	V/F 47 (43.1)	NA1/2 58 (53.2)
	T/T 7 (6.4)	H/H 78 (71.6)	V/V 9 (8.3)	NA1/1 39 (35.8)
Healthy individuals $n=183/301$ (60.8%)				
	I/I 115 (62.8)	R/R 5 (2.7)	F/F 95 (51.9)	NA2/2 24 (13.1)
	I/T 60 (32.8)	R/H 54 (29.5)	V/F 73 (39.9)	NA1/2 83 (45.4)
	T/T 8 (4.4)	H/H 124 (67.8)	V/V 15 (8.2)	NA1/1 76 (41.5)

Numbers in parentheses indicate the percentage.

<sup>a</sup>In the Japanese, *DRB1\*0101*, *0401*, *0404*, *0405*, *0410*, *1001*, *1402* and *1406* are the alleles encoding SE. Carrier frequency of SE is significantly increased in RA ( $\chi^2=70.3$ ,  $P<10^{-17}$ , odds ratio [OR]: 3.8, 95% confidence interval [CI]: 2.8–5.3).

<sup>b</sup>Significant difference of genotype distribution of *FCGR3A* was observed between patients with SE and healthy individuals with SE ( $\chi^2$  analysis from  $2 \times 3$  contingency table:  $\chi^2=6.8$ ,  $df=2$ ,  $P=0.03$ ,  $P_{\text{corr}}=0.24$ ; Armitage's test for trend:  $\chi^2=5.7$ ,  $df=1$ ,  $P=0.017$ ,  $P_{\text{corr}}=0.14$ ), and between *FCGR3A-F/F vs (F/V+V/V)* ( $\chi^2$  analysis from  $2 \times 2$  contingency table:  $\chi^2=6.8$ ,  $df=1$ ,  $P=0.009$ ,  $P_{\text{corr}}=0.072$ ).

**Table 4** Genetic interaction between *HLA-DRB1* SE and *FCGR3A-176F/F*

SE	<i>FCGR3A-F/F</i> <sup>a</sup>	RA (n=379)		Healthy Individuals (n=301)		OR <sup>b</sup>	95% CI <sup>c</sup>
+	+	151	(39.8)	49	(16.3)	4.84	3.08–7.61
+	–	119	(31.4)	69	(22.9)	2.71	1.74–4.22
–	+	53	(14.0)	95	(31.6)	0.88	0.55–1.41
–	–	56	(14.8)	88	(29.2)	1	

Percentages are shown in parentheses.

<sup>a</sup>+: *FCGR3A-F/F* genotype, -: *FCGR3A-F/V* or *V/V*.

<sup>b</sup>OR: odds ratio. OR was calculated against individuals with SE-, *FCGR3A-F/V* or *V/V* genotype.

<sup>c</sup>CI: confidence interval.

patients and controls carrying *HLA-DRB1* SE, while such a trend was not observed in patients and controls without SE. These results indicated that genetic interaction may be present between *HLA-DRB1* and *FCGR3A* genes.

Two recent papers also demonstrated association of *FCGR3A* polymorphisms with RA. Nieto *et al*<sup>7</sup> showed that *FCGR3A-176F/F* is associated with RA in the southern Spanish population. In contrast, Morgan *et al*<sup>8</sup> demonstrated association of *FCGR3A-176V* allele in UK Caucasian and North Indians/Pakistanis. Interestingly, both groups provided evidence for a genetic interaction between *FCGR3A* and *HLA-DRB1*. Therefore, although  $P_{corr}$  did not reach statistical significance, our present data showing the association of *FCGR3A-176F/F* with RA in individuals carrying SE are considered to confirm the observation by Nieto *et al*<sup>7</sup> in a different population. It is difficult to understand why a discrepancy in the associated allele is present among studies; it could be related to genetic heterogeneity, ethnic difference or technical difference.

Significant linkage disequilibria were observed between the alleles of *FCGR* gene family, which are closely located on 1q23 in the order of 2A-3A-2C-3B-2B.<sup>13</sup> However, the status of linkage disequilibrium has been shown to be different in different populations, and does not simply accord with the gene order.<sup>2</sup> Thus, one conceivable possibility was that the association of *FCGR3A* with RA might be caused by the linkage disequilibrium with another primarily associated gene, and because of the difference in the allele frequency and linkage disequilibrium, each of the *FCGR3A* alleles was detected to be associated in each population. However, our present data indicate that other *FCGR* genes are not likely to be such genes of primary significance, including *FCGR2B*, which was most intriguing in terms of functions. However, the possibility that other polymorphisms in *FCGR2B*, including recently reported promoter SNP,<sup>13</sup> might be associated with RA cannot be excluded at this stage. In addition, another recently reported gene within this chromosomal region encoding Fc receptor-related molecule might be another candidate.<sup>14</sup>

RA is characterized by the production of RF, which reacts with the Fc portion of the IgG molecule. Immune complexes containing RF can interact with FcγRs, which may contribute to the inflammation in RA. The expression of FcγRIIIA on macrophage in the synovium and dermis was suggested to be involved in the immune complex-induced tissue damage in RA.<sup>15</sup> A major role for

macrophage FcγRIIIA in the induction of tumor necrosis factor  $\alpha$  following receptor ligation by small immune complex was shown in RA.<sup>16</sup> At this point, it is not clear how FcγRIIIA-176F protein, previously shown to be associated with lower affinity to IgG1, IgG3 and reduced signaling in NK cells,<sup>1</sup> is associated with the pathogenesis of RA.

Interestingly, genetic interaction between MHC and FcγR has also been shown in the mouse model of SLE.<sup>17</sup> The molecular mechanism underlying such an interaction remains an important subject for future research. One of the likely speculations may be an effect of *FCGR3A* polymorphisms on phagocytosis and/or activation of antigen presenting cells, which express *HLA-DRB1* and present putative 'arthritogenic' antigens.

There are some limitations in this study; namely, the sex ratio and age distribution are different between the patients and controls. However, significant difference of *FCGR* genotype frequencies was not observed between male and female controls; therefore, adjustment of the male-to-female ratio between patients and controls did not affect the results (data not shown). Since the controls are younger compared with the patients, it is possible that up to 2 or 3 individuals out of 303 of controls might develop RA in the future (the estimated prevalence of RA is 1% or less in Japan). In that sense, our current study design is considered to be a conservative one, and the significant difference observed in this study would remain unchanged even if age-matched controls were employed.

In conclusion, among the *FCGR2A*, *2B*, *3A* and *3B* polymorphisms, only *FCGR3A-176F/F* genotype was associated with RA in *HLA-DRB1* SE positive subjects. These results indicated that *FCGR3A-176F*, or other allele(s) in linkage disequilibrium with it (but not *FCGR2A*, *2B* and *3B* alleles), may confer genetic risk to RA in concert with *HLA-DRB1* SE, in the Japanese.

## Patients and methods

### Subjects

A total of 382 unrelated, Japanese patients with RA and 303 unrelated, Japanese controls were examined. The group of patients with RA consisted of 40 males and 342 females between the ages of 21 and 84 (mean  $\pm$  sd 58.8  $\pm$  11.6). They were followed in the outpatient clinics of the University of Tokyo and Matsuta Clinic (Tokyo, Japan). The patients were diagnosed according to the American College of Rheumatology criteria for RA.<sup>18</sup>

Eighty three percent of the patients were positive for RF, and the majority had erosive disease.

The group of healthy individuals, 167 males and 136 females between the ages of 21 and 61 (mean  $\pm$  sd  $35.3 \pm 9.9$ ) consisted of researchers, laboratory workers and students. It should be noted that the central part of Japan has been shown to be relatively homogeneous with respect to genetic background, permitting the case-control approach employed in this study.<sup>19</sup>

This study was reviewed and approved by the research ethics committee of the University of Tokyo.

### Genotyping of *FCGR3A-F176V* polymorphism

The genomic DNA used for genotyping was purified from the peripheral blood cells of patients and healthy individuals using a QIAamp blood kit (Qiagen, Hilden, Germany).

*FCGR3A-F176V* genotyping was performed using PCR-single strand conformation polymorphism (SSCP) method. 152 bp fragment was amplified using primer set of 3A-F: 5'-TATTTACAGAATGGCAAAGG-3' and 3A-R: 5'-GTGATGGTGATGTTACAGT-3'. PCR reaction was carried out in 25  $\mu$ l reaction mixtures containing 1  $\mu$ l of genomic DNA, 0.4  $\mu$ M of each primer, 0.2 mM dNTPs, 2.5 mM MgCl<sub>2</sub> and 1 U AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems, Norwalk, CT, USA). The amplification procedure consisted of initial denaturation at 96°C for 10 min, 35 cycles of denaturation at 96°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, followed by a last extension at 72°C for 5 min. The amplified DNA fragment was applied to 10% polyacrylamide gel (acrylamide:bis = 49:1) containing 5% glycerol. Electrophoresis was carried out for 90 min at 4°C. The separated fragments were visualized with silver staining.

### Genotyping of *FCGR2B*, *2A* and *3B* polymorphisms

*FCGR2B-I232T* genotyping was carried out by nested PCR and fluorescence resonance energy transfer (FRET) technology using LightCycler™ (Roche Diagnostics, Mannheim, Germany), as described in Kyogoku *et al*<sup>2</sup> *FCGR2A-H131R* genotyping was performed using PCR-restriction fragment length polymorphism method with a mismatched primer, and *FCGR3B-NA1/2* genotyping using a PCR-preferential homoduplex formation assay and PCR-sequence specific primers method.<sup>2,9</sup>

### Genotyping of *HLA-DRB1* alleles

*HLA-DRB1* alleles were determined at the sequence level using PCR microtiter plate hybridization assay.<sup>4</sup>

### Statistical analysis

Statistical analyses for association were performed using StatView for Windows, version 5.0. (SAS Institute Inc., Cary, NC, USA).  $\chi^2$  tests were used to analyze association of the *FCGR* polymorphisms with susceptibility to RA. In addition, genotype relative risk of *FCGRs* was estimated according to Lathrop,<sup>10</sup> and Armitage's test for trend in proportions was performed according to Sasieni.<sup>11</sup> In the analysis of association of *FCGR* genotypes in the subjects stratified with respect to *HLA-DRB1*, the *P*-values were corrected for multiple comparisons (*P*<sub>corr</sub>) by multiplying the *P*-values by 8 (comparisons of four *FCGR* loci in each of SE positive and SE negative groups). Haplotype frequencies were estimated using EH program.<sup>12</sup>

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