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Single-nucleotide polymorphisms and copy number variations of the *FCGR2A* and *FCGR3A* genes in healthy Japanese subjects

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Abstract. FcγRII and FcγRIII are low-affinity Fcγ receptors that are encoded by the *FCGR2A* and *FCGR3A* genes, respectively. These genes contain functional single-nucleotide polymorphisms (SNPs), which alter the binding affinities of these receptors for the γ chain of the Fc fragment of immunoglobulin G. The known SNPs in *FCGR2A* and *FCGR3A* are rs1801274 (A>G; H131R) and rs396991 (T>G; F158V), respectively. It is also known that there are copy number variations (CNVs) in the genetic locus (1q23) where *FCGR2A* and *FCGR3A* are located. However, the frequencies of these SNPs and CNVs have not been determined in the Japanese population. The aim of this study was to investigate SNPs and CNVs in *FCGR2A* and *FCGR3A* among 113 healthy individuals. The SNPs and CNVs in *FCGR2A* and *FCGR3A* were determined using the TaqMan® SNP Genotyping and the TaqMan® Copy Number assays. Our results revealed that the incidence of *FCGR2A* (rs1801274) genotypes were as follows: A/A, 69.9%; A/G, 29.2%; and G/G, 0.9%. The incidence of the *FCGR3A* (rs396991) genotypes were as follows: T/T, 56.7%; T/G, 38.9%; and G/G, 4.4%). No CNVs were detected for *FCGR2A*. To the best of our knowledge, this finding has not been previously reported in the Japanese population. By contrast, CNVs were observed in *FCGR3A* (3 subjects were found to harbour a gene deletion and 5 subjects had 3 copies of the gene). Using simple commercially available assays we were able to confirm previous findings regarding *FCGR2A* and *FCGR3A* alleles and CNVs. These assays may provide a basis for the investigation of the role of these genes in the efficacy of antibody-based drugs, such as trastuzumab and rituximab, in Japanese subjects.

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Introduction

Fcγ receptors (FcγRs) bind specifically to the γ chain of the Fc fragment of immunoglobulin G (IgG) and are located on the surface of immune cells, such as monocytes, macrophages and natural killer cells. FcγRs are directly involved in the function of these immune cells and regulate immune responses. There are 3 subtypes of FcγRs, i.e., FcγRI, FcγRII and FcγRIII, which are highly homologous to one another. FcγRI binds to IgG with high-affinity, whereas FcγRII and FcγRIII are low-affinity receptors for IgG (1,2). Each of these receptors has multiple isoforms. Among these isoforms, FcγRIIa and FcγRIIIa are encoded by the *FCGR2A* and *FCGR3A* genes, respectively. It is also known that there are non-synonymous single-nucleotide polymorphisms (SNPs) for these genes, including rs1801274 (A>G; H131R) for *FCGR2A* and rs396991 (T>G; F158V) for *FCGR3A* (3-7). These SNPs encode for functional receptors and the receptor encoded by each variant gene has a distinct binding affinity for the Fc fragment of an antibody. It was previously reported that the affinity of FcγRIIa with the H131 variant is higher compared to that with the R131 variant and the affinity of FcγRIIIa with the V158 variant is higher compared to that with the F158 variant (4,8,9). It was also reported that these SNPs are associated with a risk for the development of autoimmune diseases, such as systemic lupus erythematosus (SLE) (10-12), and may also be involved in individual differences in the efficacy of antibody-based drugs, such as rituximab (13-16), trastuzumab (17,18) and cetuximab (19-21). However, there is currently no consensus on these findings and available information regarding the frequency of these SNPs in the Japanese population has not been adequately investigated (7).

In addition, it was recently demonstrated that copy number variations (CNVs) may also be involved in disease susceptibility (22-24). It is known that there are CNVs at the genetic locus (1q23) where *FCGR2A* and *FCGR3A* are located (6,25-28). It was also reported that CNVs are a risk factor for the development of autoimmune diseases, such as SLE (9,26,29,30). However, compared with SNPs for these genes, the involvement of CNVs in *FCGR2A* and *FCGR3A* and the risk of development of autoimmune diseases has not been adequately investigated. Although CNVs in *FCGR3A* have been reported previoulsy, there is no available information regarding the frequency of CNVs in *FCGR2A* in the Japanese population.

The aim of this study was to investigate the prevalence of SNPs and CNVs in *FCGR2A* and *FCGR3A* in the Japanese population, in order to aid the future diagnosis and treatment of autoimmune diseases and the prediction of antibody-based drug efficacy.

Materials and methods

Subjects. A total of 113 healthy Japanese volunteers (59 males and 54 females), aged 21-41 years and residing in the city of Sapporo and neighboring areas, were enrolled in this study. The study protocol was approved by the Institutional Review Board of the National Hospital Organization Hokkaido Cancer Center and written informed consent was obtained from all participating subjects.

Genomic DNA isolation. Genomic DNA was isolated from peripheral blood anticoagulated with K_2-EDTA using a Puregene® DNA Isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The quantity and quality of the extracted genomic DNA were evaluated using a NanoDrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and electrophoresis with an ethidium bromide-agarose gel, respectively.

Genotyping of SNPs. The genotypes of rs1801274 (A>G) in the *FCGR2A* gene and rs396991 (T>G) in the *FCGR3A* gene were determined by the TaqMan® SNP Genotyping assay (Life Technologies, Carlsbad, CA, USA). The assay IDs were c_9077561_20 for rs1801274 and c_25815666_10 for rs396991. The polymerase chain reaction (PCR) mixture contained 5 μl of 2X TaqMan Fast Universal PCR Master mix (Life Technologies) for rs1801274 and 5 μl of 2X TaqMan Universal PCR Master mix (Life Technologies) for rs396991. Both reactions used ~100 ng of genomic DNA and 0.25 μl of 40X TaqMan SNP Genotyping assay, with the final volume adjusted to 10 μl using sterile purified water. The cycling conditions were as follows: rs1801274 was pre-warmed at 60˚C for 1 min, followed by AmpliTaq Gold activation (hot start) at 95˚C for 20 sec, denaturation at 95˚C for 3 sec, annealing/extension at 60˚C for 30 sec, repeated for 40 cycles, followed by a post-read at 60˚C for 1 min; rs396991 was pre-warmed at 60˚C for 1 min, followed by uracil-DNA glycosylase activation at 50˚C for 2 min, AmpliTaq Gold activation (hot start) at 95˚C for 10 min, denaturation at 95˚C for 15 sec, annealing/extension at 60˚C for 1 min, repeated for 40 cycles and followed by a post-read at 60˚C for 1 min.

Table I. *FCGR2A* (rs1801274; A>G) and *FCGR3A* (rs396991; T>G) genotypes of the subjects included in this study.

Gene	SNP	Genotype	No. of subjects (%)
FCGR2A	rs1801274	A/A	79 (69.9)
		A/G	33 (29.2)
		G/G	1 (0.9)
FCGR3A	rs396991	T/T	64 (56.7)
		T/G	44 (38.9)
		G/G	5 (4.4)

SNP, single-nucleotide polymorphism.

Following amplification of the DNA templates using the ABI PRISM® 7500 Fast Real-Time PCR system (Life Technologies), allele determinations were performed with endpoint fluorescence intensity measurements, using the 7500 System software (Life Technologies).

Determination of CNVs. For the determination of CNVs at the *FCGR2A* and *FCGR3A* loci, the TaqMan Copy Number assay (Life Technologies) was used. The assay IDs were Hs00103511_cn for *FCGR2A* (exon 1-intron 1) and Hs00139300_cn for *FCGR3A* (exon 2). In addition, TaqMan Copy Number Reference assay RNase P (Life Technologies) was used. The PCR amplifications were performed in a reaction volume of 20 μl, containing 10 μl of 2X TaqMan Universal PCR Master mix, 1.0 μl of 20X TaqMan Copy Number assay, 1.0 μl of 20X TaqMan Copy Number Reference assay and ~20 ng of template DNA. The cycling conditions were as follows: enzyme activation (hot start) at 95˚C for 20 sec, followed by denaturation at 95˚C for 15 sec, annealing/extension at 60˚C for 1 min and repeated for 40 cycles. The copy numbers were determined using CopyCaller™ software (Life Technologies) following amplification of the DNA templates using the ABI PRISM® 7500 Fast Real-Time PCR system and measuring fluorescence intensity at the endpoint. To obtain an accurate copy number, 4 different DNA templates were used for each sample. At least 7 samples were simultaneously analyzed to determine the copy number using CopyCaller™ software (Invitrogen Life Technologies, Carlsbad, CA, USA).

Statistical analysis. The Hardy-Weinberg equilibrium (HWE) test for assessing the rs1801274 and rs396991 genotype frequency among the subjects was conducted using PowerMarker software, version 3.0 (31) and a HWE calculator including analysis for ascertainment bias software (32). The allele and CNV frequencies were compared with previously published data from healthy individuals using the Fisher's exact test with SPSS Statistics software, version 21 (IBM Japan, Ltd., Tokyo, Japan). A two-tailed $P<0.05$ was considered to indicate a statistically significant difference.

Results

Genotype determinations and comparisons of allele frequencies with published data on healthy individuals. The genotype

Table II. Ethnic differences in the allele frequency of *FCGR2A* rs1801274 (A>G) in healthy subjects.

Study	Population	No.	Allele frequency		P-value[a]	Refs.
			A	G		
The present study	Japanese	113	0.845	0.155	-	
Iwasaki *et al*	Japanese	403	0.800	0.200	0.128	(7)
	Japanese Brazilians	80	0.806	0.194	0.318	
	Non-Japanese Brazilians	386	0.469	0.531	<0.0001	
Breunis *et al*	Northern-European	100	0.540	0.460	<0.0001	(6)
Van Den Berg *et al*	Ethiopians	77	0.470	0.530	<0.0001	(5)
	Norwegians	86	0.420	0.580	<0.0001	
Reilly *et al*	African American	50	0.440	0.560	<0.0001	(3)
	Caucasian American	47	0.540	0.460	<0.0001	

[a]The Fisher's exact test was used to determine the differences between the allele frequency in this study and previously published results.

Table III. Ethnic differences in the allele frequency of *FCGR3A* rs396991 (T>G) in healthy subjects.

Study	Population	No.	Allele frequency		P-value[a]	Refs.
			T	G		
The present study	Japanese	113	0.761	0.239	-	
Iwasaki *et al*	Japanese	403	0.754	0.246	0.824	(7)
	Japanese Brazilians	80	0.713	0.287	0.300	
	Non-Japanese Brazilians	386	0.715	0.285	0.177	
Breunis *et al*	Northern-European	100	0.710	0.290	0.232	(6)
Van Den Berg *et al*	Ethiopians	77	0.540	0.460	<0.0001	(5)
	Norwegians	86	0.660	0.340	0.031	

[a]The Fisher's exact test was used to determine the differences between the allele frequency in this study and other previously published results.

distributions for rs1801274 of *FCGR2A* and rs396991 of *FCGR3A* obtained in this study are presented in Table I. Divergence from the HWE was not observed for either SNP.

Comparisons of the frequencies for each SNP allele with previously published data are shown in Table II for rs1801274 and in Table III for rs396991. The minor allele frequency (MAF) for rs1801274 obtained in this study (0.155) was significantly lower among Japanese individuals compared to that among other ethnic groups (Table II). The MAF for rs396991 (0.239) was marginally different from that for rs1801274 (0.155). These frequencies were not significantly different from those reported for other Japanese populations. However, the allele frequencies for rs396991 obtained in this study for Japanese subjects were significantly different from those observed in Ethiopians and Norwegians (Table III). In particular, the T allele frequency among Japanese was higher compared to that among Ethiopians and Norwegians.

CNV determination and comparisons of CNV frequencies with published data on healthy individuals. The CNVs for *FCGR2A* and *FCGR3A* from this study are presented in Table IV. There was no CNV detectable for *FCGR2A*, as all subjects tested had 2 copies of this gene.

However, with regard to *FCGR3A*, 5 subjects had 3 copies of this gene and 3 subjects had a single copy (gene deletion). The associations between CNVs in *FCGR3A* and the rs396991 genotypes were as follows: 3 subjects with a single copy of *FCGR3A* had the T/T genotype and of the 5 subjects with 3 gene copies, 1 had the T/T and 4 had the T/G genotype. Comparisons between the CNVs in *FCGR3A* from this study and those from previously published studies are shown in Table V. There were no significant differences between our results and those of previously published studies.

Discussion

In this study, the MAF for rs1801274 (A>G, H131R) of *FCGR2A* was 0.155 and only 1 subject (0.9%) had the G/G genotype. A previous study indicated that the MAF of *FCGR2A* among Japanese and Japanese-Brazilians was 0.200 and 0.194, respectively (7). Therefore, the SNP frequency for this minor allele appears to be between 0.15-0.2 in the

Table IV. Number of copy number variations in *FCGR2A* and *FCGR3A* in this study.

Gene	TaqMan® Copy Number assay ID	Copy number variation (%)				
		0 copies	1 copy	2 copies	3 copies	4 copies
FCGR2A	Hs00103511_cn	0	0	113 (100)	0	0
FCGR3A	Hs00139300_cn	0	3 (2.7)	105 (92.9)	5 (4.4)	0

Table V. Ethnic differences in the frequency of copy number variation in *FCGR3A* in healthy subjects.

Study	Population	No.	Copy number variation frequency				P-value[a]	Refs.
			1 copy	2 copies	3 copies	4 copies		
The present study	Japanese	113	0.027	0.929	0.044	0	-	
Hollox *et al*	Japanese	32	0	0.875	0.094	0.031	0.301	(25)
	Chinese	32	0.031	0.813	0.156	0	0.082	
	Yoruba	35	0.029	0.943	0.029	0	1	
	Europeans	110	0.027	0.891	0.082	0	0.356	
Breunis *et al*	Northern-Europeans	129	0.016	0.946	0.039	0	0.606	(26)
Zhou *et al*	Han Chinese	146	0.041	0.877	0.075	0	0.289	(27)

[a]Fisher's exact test. The differences in the copy number distributions between this study and other studies were determined by dividing the subjects into 2 groups: group A with 2 gene copies and group B with copy numbers other than 2.

Japanese population. The rs1801274 allele frequency among the Japanese subjects in this study was significantly different from those previously reported among Europeans (3-6) and Africans (3,4). Therefore, there are ethnic differences in rs1801274 SNP frequency. The MAF for rs396991 (T>G, F158V) of *FCGR3A* was previously reported to be 0.2-0.3 among Japanese individuals (7). We confirmed this frequency in a separate population. Therefore, the prevalence of this SNP frequency was found to be marginally higher compared with that of *FCGR2A* rs1801274. The MAF of rs36991 was found to be significantly lower in Japanese compared with that in Ethiopians (P<0.0001) and marginally lower compared with that observed in Norwegians (P=0.031) (4). Since rs1801274 and rs396991 are both functional SNPs, it may be predicted that the affinities of FcγIIa and FcγIIIa for antibodies may display ethnic variation.

No CNVs were detected for *FCGR2A*. To the best of our knowledge, this was the first study to assess CNVs of this gene in Japanese individuals. However, Breunis *et al* (26) also failed to detect any CNVs in *FCGR2A* among healthy European subjects, as well as among patients with diseases such as idiopathic thrombocytopenia, Kawasaki disease, or rheumatoid arthritis. By contrast, CNVs in *FCGR3A* were identified [3 subjects with a gene deletion (single copy) and 5 subjects with 3 gene copies]. This finding confirmed the previously observed presence of CNVs in *FCGR3A* (25). Unlike that previous small study, we did not identify any individuals with 4 gene copies; however, this may be due to the low frequency of this event. We observed a similar incidence of *FCGR3A* CNVs between the subjects in this study and Chinese (25,27), European (25,26) and African (25,26) subjects. Therefore, it appears that CNVs in *FCGR3A* are a common finding across different ethnic groups. Changes in gene dosage caused by CNVs may result in individual variations of the FcγRIIIa expression.

Three subjects with a single *FCGR3A* copy were found to have an rs396991 T/T genotype. However, their actual genotypes were considered to be T/- (T/del) when CNVs were taken into consideration. It was also predicted that the actual genotype of 1 subject was T/T/T and the 4 subjects genotyped as T/G were either T/T/G or T/G/G. Therefore, it is recommended to take CNVs for *FCGR3A* into consideration when evaluating the SNP genotype.

The TaqMan Copy Number assay to determine CNVs in *FCGR2A* and *FCGR3A* used a probe for *FCGR2A* that recognized the region between exon 1 and intron 1 and the probe for *FCGR3A* recognized a region of exon 2. To confirm our results, additional CNV analysis, using multiple probes that are able to recognize various regions of each gene should be considered. Additional analysis of the genotypes of *FCGR2B*, *FCGR2C* and *FCGR3B*, which are linked in tandem to *FCGR2A* and *FCGR3A* at 1q23, should also be considered in future studies.

Using commercially available TaqMan-based assays, we determined the incidence of SNPs and CNVs in *FCGR2A* and *FCGR3A* among healthy Japanese individuals. These assays confirmed data previously reported by studies that utilized more complex methods, such as multiplex ligation-dependent probe amplification (26). However, a large-scale analysis of the entire *FCGR* region, using a next-generation genome sequencing, may be useful for a more detailed investigation of genetic variability of *FCGR* in the Japanese population.

The straightforward methods used in this study to obtain information regarding the *FCGR* SNPs and CNVs among Japanese subjects may allow the development of diagnostic methods and treatments for autoimmune diseases, including SLE, as well as genetic biomarker research to predict the efficacy of antibody-based drugs, such as trastuzumab and rituximab.

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