FCGR3B polymorphism in three ethnic Chinese populations

L. YAN, F. ZHU, L. JIN, Q. LV, AND Q. FU

FcγRIIIb receptor is expressed primarily on neutrophils as three polymorphic antigens (HNA-1a, HNA-1b, and HNA-1c) that are encoded by alleles *FCGR3B*1, FCGR3B*2,* and *FCGR3B*3,* respectively. These antigens play an important role in immune neutropenia; their absence predisposes individuals who lack them to life-threatening infections. This study investigated the *FCGR3B* gene frequencies in three ethnic Chinese populations: Han, She, and Tajik. *FCGR3B*1, FCGR3B*2,* and *FCGR3B*3* were genotyped by PCR using sequence specific primers (PCR-SSP). The results showed the gene frequencies were 0.55 for *FCGR3B*1* and 0.45 for *FCGR3B*2* in 177 Han individuals, 0.69 for *FCGR3B*1* and 0.31 for *FCGR3B*2* in 87 She individuals, and 0.35 for *FCGR3B*1* and 0.65 for *FCGR3B*2* in 99 Tajik individuals, respectively. The $FCGR3B_{null}$ genotype was not found, but the *FCGR3B*3* allele was identified in only three individuals in the Tajik population. DNA clone and sequencing confirmed that these individuals had the C⇒A mutation at position 266 on exon 3. This study found that the gene frequencies in Han and She ethnic groups were similar to those previously reported in the Asian population, but the *FCGR3B* allele frequencies in the Tajik population were more similar to that of Caucasians. ***Immunohematology* 2005;21:25–28.**

Key Words: *FCGR3B,* gene frequency, Chinese population, PCR-SSP, Fc receptor

FcγRIII is a low-affinity Fc gamma receptor on leukocytes, existing as two isoforms, FcγRIIIa and FcγRIIIb.[1] FcγRIIIa is expressed on natural killer cells, monocytes, and macrophages. FcγRIIIb is expressed primarily on neutrophils and as three polymorphic antigens (human neutrophil antigen, HNA-1a [formerly NA1]), HNA-1b (formerly NA2), and HNA-1c (formerly SH, NA3), which are encoded by alleles *FCGR3B*1, FCGR3B*2,* and *FCGR3B*3,* respectively.[2] *FCGR3B*1* and *FCGR3B*2* differ at five nucleotide (nt) positions (141G⇒C, 147C⇒T, 227A⇒G, 277G⇒A, and 349G⇒A) that encode four amino acid changes.[1,2] *FCGR3B*3* is identical to *FCGR3B*2* except for a single base change (266C⇒A) that encodes amino acid Ala78Asp.[3]

Polymorphisms of neutrophil antigens are important clinically because of their role in antibody-mediated immune neutropenia. In addition, alleles of *FCGR3B* distinguish certain racial and ethnic populations. In French, German, American Caucasian, and African Black populations, *FCGR3B*2* is the most prevalent *FCGR3B* allele,[4,5,6] whereas in certain Chinese and Japanese populations, *FCGR3B*1* is more prevalent.[7,8] *FCGR3B*3* occurs in 0.05 percent of Caucasians,[3] but has not been reported in Asians.[7,8] Rarely, individuals lack FcγRIIIb neutrophil antigens (NA_{null}), predisposing them to life-threatening infections. Also, several low-prevalence *FCGR3B* variant alleles that carry a single-base substitution of one of the five polymorphic sites on *FCGR3B* have been identified in certain Caucasian and Black populations.[5,9]

Frequencies of *FCGR3B* genes and of seven *FCGR3* variants have been described in Chinese individuals from Zhejiang Province.[8] China's population is large and genetically heterogeneous. Most indigenous Chinese are ethnically Han (92%). The remaining 8 percent of indigenous Chinese represent 55 ethnic minorities. Ethnic She (630,000 individuals) reside in Fujian, Zhejiang, and Jiangxi Provinces. Ethnic Tajik (33,000 individuals) reside in Xinjiang Province. We describe the frequencies of *FCGR3B* alleles and the results of screening for FcγRIII variants among Han, She, and Tajik populations.

Materials and Methods

Subjects

Peripheral blood samples were collected from volunteer blood donors after obtaining informed consent. We interviewed subjects to categorize them as Han (Zhejiang Province), She (Zhejiang Province), or Tajik (Xinjiang Province).

DNA extraction and genotyping FCGR3B

We extracted genomic DNA from whole blood, using a QIAamp Blood Kit (Qiagen GmbH, Hilden,Germany). Allele-specific DNA amplification was performed according to the protocol of Bux et al.[3,10] Primer sequences are listed in Table 1.[3] Genotypes were assigned by the presence or absence

Table 1. Primers for PCR-SSP and DNA sequencing

Name	Primer sequence
FCGR3B*1 sense	5´cag tgg ttt cac aat gtg aa3´
FCGR3B*1 antisense	5´atg gac ttc tag ctg cac 3´
FCGR3B*2 sense	5´caa tgg tac agc gtg ctt 3´
FCGR3B*2 antisense	5´atg gac ttc tag ctg cac 3´
FCGR3B*3 sense	5´aag atc tcc caa agg ctg tg 3´
FCGR3B*3 antisense	5´tct gtc gtt gac tgt gtc at 3´
Exon 3 forward	5´tgagctcattctggctttga3´
Exon 3 reverse	5´tcaggacccttttgtttcacc3´

of an electrophoretic band in 2% agarose gel stained with ethidium bromide and visualized by ultraviolet light (GeneGenius, Syngene, UK).

Sequencing FCGR3A and FCGR3B

We performed DNA sequencing on samples from 12 individuals from the Tajik population with three different *FCGR3B* forms. DNA fragments encompassing the full exon 3 coding region were amplified using primers designed by our laboratory (Table 1), which can amplify both *FCGR3A* and *FCGR3B* genes. The PCR reaction mixture contained 50 to 100 ng genomic DNA, 2.5 µL of dNTP (200 µmol/L each), 2.5 µL 10X PCR buffer (100 mmol/L Tris-HCl, pH 8.3, and 500 mmol/L KCl), 2.0 mmol/L MgCl2, 0.5 µmol/L of forward and reverse primers, and 1.0 U Taq DNA polymerase (Roche company) in a final volume of 25 µL. PCR amplification was performed with initial denaturing at 95°C for 5 minutes followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, plus a final extension at 72°C for 10 minutes. DNA amplified fragments were cloned using TOPO cloning sequencing kits (Invitrogen Co., Carlsbad, CA) according to manufacturer's instructions. Positive clones according to blue and/or white selection were cultured in LB medium. Plasmid DNA was extracted by using a plasmid DNA purification kit (Shanghai Bocai, China). DNA sequences were determined by amplified primers, using cycle sequencing kits (BigDye Terminator, Applied Biosystems, Foster City, CA) and a Genetic Analyzer (ABI Prism 377, Applied Biosystems), according to the manufacturer's instructions.

Calculation of allele frequencies and statistical analysis

Allele frequencies were calculated using the formula of Steffensen et al.[6] Briefly, *FCGR3B*1* and *FCGR3B*2* were treated as two existing alleles at the same locus. Based on this assumption, the frequencies were calculated independently for *FCGR3B*3* by counting the number of each allele and calculating the percentage. *FCGR3B*3* frequency was calculated as one-half of the percentage of allele-positive individuals. We used chi-squared analyses to test for Hardy-Weinberg equilibrium for *FCGR3B* genes. A $p < 0.05$ was considered to be statistically significant.

Results

Allele-specific DNA amplification

Among the 172 Hans, 87 (50.6%) genotyped as *FCGR3B*1+,*2+,*3-;* 34 (19.8%) genotyped as *FCGR3B*1-,*2+,*3-;* and 51 (29.6%) genotyped as *FCGR3B*1+,*2-,*3-* (Table 2). Allele frequencies were 0.55 for *FCGR3B*1,* 0.45 for *FCGR3B*2,* and 0.00 for *FCGR3B*3* (Table 3). Among the 87 Shes, 36 (41.4%) individuals genotyped as *FCGR3B*1+,*2+, *3-;* 9 (10.3%) genotyped as *FCGR3B*1-,*2+,*3-;* and 42 (48.3%) genotyped as *FCGR3B*1+,*2-,*3-.* Allele frequencies were 0.69 for *FCGR3B*1,* 0.31 for *FCGR3B*2,* and 0.00 for *FCGR3B*3.* Among the 99 Tajiks, 48 (48.5%) genotyped as *FCGR3B*1+,*2+,*3-;* 38 (38.4%) genotyped as *FCGR3B*1-,*2+,*3-;* 10 (10.1%) genotyped as *FCGR3B*1+,*2-,*3-;* 1 (1%) individual genotyped as *FCGR3B*1+,*2+,*3+;* and 2 (2%) individuals genotyped as *FCGR3B*1-,*2+,*3+.*

Table 2. Genotype frequencies observed in the Han, She, and Tajik populations

	Han		She		Tajik	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**
*FCGR3B*1+,*2+,*3-*	87	50.6	36	41.4	48	48.5
*FCGR3B*1-,*2+,*3-*	34	19.8	9	10.3	38	38.4
*FCGR3B*1+,*2-,*3-*	51	29.6	42	48.3	10	10.1
*FCGR3B*1-,*2-,*3-*	0	0.0	0	0.0	0	0.0
*FCGR3B*1+,*2+,*3+*	0	0.0	0	0.0	1	1.0
*FCGR3B*1-,*2+,*3+*	0	0.0	0	0.0	2	2.0
*FCGR3B*1+,*2-,*3+*	0	0.0	0	0.0	0	0.0
*FCGR3B*1-,*2-,*3+*	0	0.0	0	0.0	0	0.0

Table 3. *FCGR3B* allele frequencies in different populations

Population	n	*FCGR3B*1*	*FCGR3B*2*	*FCGR3B*3*
Han(Zhejiang)	172	0.55	0.45	0.00
She minority	87	0.69	0.31	0.00
Tajik minority	99	0.348	0.652	0.015
Japanese[7]	400	0.622	0.378	0.00
Danish[6]	200	0.365	0.635	0.030
Ugandan[5]	43	0.395	0.558	0.174
Northern German[5]	260	0.373	0.627	0.025

Allele frequencies were 0.348 for *FCGR3B*1,* 0.652 for *FCGR3B*2,* and 0.015 for *FCGR3B*3.* Differences between *FCGR3B*1, FCGR3B*2,* and *FCGR3B*3* frequencies among Hans, Shes, and Tajiks were significant ($p < 0.001$). We did not find the *FCGR3B*1–,*2-,*3–* (*FCGR3B*$_{null}$) genotype among the 358 samples tested. Three Tajik individuals expressed the *FCGR3B*3* allele. Table 3 also lists previously published allele frequencies in certain other populations.

Table 4. *FCGR3* variants at nucleotide positions within exon 3 from 12 Tajik subjects

FCGR3 form	Number of individuals	Number of clones	Nucleotide positions 141	147	227	266	277	349
FCGR3A	12	35	G	C	G	C	G	A
Variant 2	2	2	G	C	G	C	A	A
*FCGR3B*1*	7	10	G	C	A	C	G	G
*FCGR3B*2*	9	15	C	T	G	C	A	A
Variant 1	3	7	C	T	G	C	G	A
Variant 3	1	1	G	T	G	C	A	A
*FCGR3B*3*	3	6	C	T	G	A	A	A

FCGR3 sequencing

DNA cloning and sequencing confirmed the results of PCR-SSP analysis by showing that three *FCGR3B*3* positive samples had a C⇒A mutation at position 266 on exon 3. We sequenced 76 clones of exon 3 of *FCGR3* from 12 Tajik subjects (Table 4). We identified three individuals (7 clones) as having *FCGR3B*2* variants (141C, 147T, 227G, 266C, 277G, 349A), two individuals (2 clones) as having *FCGR3A* variants (141G, 147C, 227G, 266C, 277A, 349A), and one individual (1 clone) as having *FCGR3B*2* variant (141G, 147T, 227G, 266C, 277A, 349A). Each variant demonstrated either a single base substitution of one of the six polymorphic sites of the *FCGR3* gene or a new combination of nucleotides at the corresponding position.

Discussion

Our results confirm that *FCGR3B*1* is more prevalent than *FCGR3B*2* among Chinese Hans and Shes, as reported previously.[7,8] We also found that *FCGR3B* allele frequencies in the Tajik population were very similar to those of Caucasians.[5,6] We identified three Tajik individuals with the *FCGR3B*3* allele, which is the first time this allele has been identified in indigenous Asians. We did not find *FCGR3B*3* in our Han or She populations, which was consistent with previous observations in other Asian populations. We also did not find the *FCGR3B*$_{null}$ genotype in any of the three Chinese populations tested, but larger scale testing is required to ascertain that it is totally absent in the Chinese population.[8]

Several *FCGR3B* variants have been described in African Blacks and American Caucasians.[9] Our finding of three new *FCGR3B* variants demonstrates that such variant genes also exist in Chinese populations. It is not known whether these variants reflect somatic mutations or multiple gene loci. Also unknown is what impact mutations resulting in variant genes have on the function of *FCGR3B* gene-encoded neutrophil antigens or their corresponding Fc gamma receptors.

References

1. Qiu WQ, de Bruin D, Brownstein BH, et al. Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. Science 1990;248:732-5.
2. Bux J. Nomenclature of granulocyte alloantigens. Transfusion 1999;39:662-3.
3. Bux J, Stein EL, Bierling P, et al. Characterization of a new alloantigen (SH) on the human neutrophil Fc gamma receptor IIIb. Blood 1997;89(3): 1027-34.
4. Kissel K, Hofmann C, Gittinger FS, et al. HNA-1a, HNA-1b, HNA-1c (NA1, NA2, SH) frequencies in African and American Blacks and in Chinese. Tissue Antigens 2000;56:143-8.
5. Flesh BK, Doose S, Siebert R, et al. FCGR3 variants and expression of human neutrophil antigen-1a, -1b, -1c in the population of northern Germany and Uganda. Transfusion 2002;42:469-75.
6. Steffensen R, Gulen T, Varming K, et al. FcγRIIIB polymorphism: evidence that NA1/NA2 and SH are located in two closely linked loci and that the SH allele is linked to the NA1 allele in the Danish population. Transfusion 1999;39:593-8.
7. Fujiwara K, Watanabe Y, Mitsunaga S, et al. Determination of granulocyte specific antigens on neutrophil Fcγreceptor IIIb by PCR preferential homoduplex formation assay, and gene frequencies in Japanese population. Vox Sang 1999;77:218.
8. Yin T, Jie J, Lixing Y, et al. *FCGR3B* gene frequencies and *FCGR3* variants in a Chinese population from Zhejiang Province. Ann Hematol 2003;82:574-8.

9. Matsuo K, Procter J, Stroncek D. Variants in genes encoding neutrophil antigens NA1 and NA2. Transfusion 2000;40:645-53.
10. Bux J, Stein EL, Santoso S, et al. NA gene frequencies in the German population, determined by polymerase chain reaction with sequence-specific primers. Transfusion 1995;35: 54-7.

Lixing Yan, MD; Faming Zhu, MD; Lei Jin, BA; Qinfeng Lv, BA; and Qihua Fu, PhD, Institute of Blood Transfusion Medicine, Blood Center of Zhejiang Province, Hangz, Zhejiang, 310006 P. R. China.