Brief communication

Variation in the *FcγR3B* gene among distinct Brazilian populations

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Abstract: The *FCGR3B* gene codes for the FcγR3b receptor, which occurs in three polymorphic forms representing the human neutrophil antigens (HNA)-1a, HNA-1b, and HNA-1c. The alleles that code for these antigens are *FCGR3B*1*, *FCGR3B*2*, and *FCGR3B*3*, respectively. New variants of these alleles have been recently described. In order to study the frequency of these alleles and the occurrence of variant forms, we sequenced part of the *FCGR3B* gene in 149 individuals belonging to four distinct Brazilian populations, i.e., 60 Amerindians, 30 Whites of European descent, 30 Afro-Brazilians, and 30 Japanese. The *FCGR3B*1* allele showed high frequency among Amerindians (0.850), with the value detected representing the highest frequency described thus far for this allele in population studies. Its frequency was 0.660 in the Japanese population studied, a value equal to that observed in Afro-Brazilians (0.600) and higher than that observed in Whites (0.480). The *FCGR3B*3* allele was only found among Afro-Brazilians, where it occurred at a frequency of 0.080, which was lower than the frequency observed among Afro-North Americans (0.207) and Ugandans (0.166). Two variant haplotypes were detected among Amerindians and Afro-Brazilians, occurring in six individuals (four Amerindians and two Afro-Brazilians). The variant haplotype *FCGR3B*1 A227G*, which occurred in homozygosis in two Amerindians and in heterozygosis in two Afro-Brazilians, is described for the first time in the present report. In general, these data reveal variability in the frequency of alleles of the *FCGR3B* gene compared to other populations of the same genetic background in other regions of the world.

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Receptors with low affinity for the Fc portion of IgG (FcγR) are predominantly expressed in hematopoietic cells and play an important role in the interaction of humoral and cell immunity (1). FcγR are divided into three classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (2). The Fcγ receptor RIII exists in two isoforms coded by two distinct, but highly homologous, genes located on chromosome 1, q22 band (3). The *FCGR3A* gene codes for the FcγRIIIa receptor, which is a transmembrane protein present on the surface of natural killer cells, monocytes and macrophages. The *FCGR3B* gene codes for the FcγRIIIb receptor which is bound by a glycosylphosphatidylinositol anchor to the neutrophil surface (4). The Fcγ

receptor RIIIb presents three variant forms denoted human neutrophil antigens (HNA)-1a, HNA-1b, and HNA-1c, previously called NA-1, NA-2, and SH, respectively. These antigens are coded by alleles *FCGR3B*1*, *FCGR3B*2*, and *FCGR3B*3*, respectively. The *FCGR3B*1* allele (HNA-1a) differs from the *FCGR3B*2* allele (HNA-1b) in five nucleotides (positions 141, 147, 227, 277, and 349), with a consequent change in four amino acids (positions 36, 65, 82, and 106). The base change at position 147 is silent. The *FCGR3B*3* allele (HNA-1c) is identical to *FCGR3B*2* except for a C→A substitution at position 266 which determines the amino acid change Ala78Asp (5–8).

Matsuo et al. (9) identified variant forms of the *FCGR3B* alleles in Japanese, Afro-American and White individuals in the United States. Using cloning and sequencing, they described a variant form of the *FCGR3B*1* allele (HNA-1a) and four variant forms of the *FCGR3B*2* allele (HNA-1b). They also identified a variant that could not be clearly classified as being an allele of the *FCGR3A* or *FCGR3B* gene. Flesch et al. (10), in a study of blood donors from northern Germany and from Uganda, detected five variant forms of the *FCGR3B*1* allele and the *FCGR3A* gene. These new alleles are due to base substitutions in one of the five polymorphic sites of the *FCGR3B* gene. The alleles closest to *FCGR3B*2* (HNA-1b) occur at higher frequency in black populations than in White or Japanese populations.

The *FCGR3A* and *FCGR3B* genes present a high degree of homology, a fact that impairs the identification of the polymorphisms described because four allelic forms are involved. Countless techniques have been proposed to clarify this point and the allele-specific polymerase chain reaction is the one most extensively used (11, 12). In the present study, we used genomic DNA sequencing in combination with allele-specific PCR primers to determine the frequency of the alleles *FCGR3B*1* (HNA-1a), *FCGR3B*2* (HNA-1b), and *FCGR3B*3* (HNA-1c) in Amerindians and in other Brazilian populations.

The Amerindian samples consisted of 60 individuals belonging to seven tribes from the Brazilian Amazon: four Arara (Karib language), nine Yanomama (Ninan language), 16 Kayapó (Jê language), 10 Wayana-Apalai (Karib language), 11 Wayampi (Tupi-Guarani language), and 10 Awá-Guajá (Tupi language). The individuals studied were apparently unrelated. Additional data for the genetic characterization of these groups have been published previously (13–15). Three additional populations were studied: 30 Whites (mostly of western and southern European ancestry), 30 Afro-Brazilians (excluding Mulattos), and 29 Japanese. Only individuals who reported the absence of racial admixture for their four grandparents were included.

Genomic DNA was extracted from peripheral blood buffy coat using the phenol-chloroform method. The DNA fragment from base number 95 to 348 of the *FCGR3B* gene was amplified by PCR using the following set of primers: 4NA 5′-AAG ATC TCC CAA AGG CTG TGG-3′ (position 95–115) and 5NA 5′ATG GAC TTC TAG CTG CAC-3′ (position 331–348). The bases were numbered according to the sequence deposited in GenBank under number X52645. The reaction was performed using 12.5 pmol of each primer, 1.25 mM dNTPs, and 0.4 units of Taq polymerase in a final volume of 50 µl. Thirty-five amplification cycles were performed according to the following protocol: 94°C for 60 s, 57°C for 60 s, and 72°C for 60 s.

Thirty-one samples (one Amerindian, 24 Afro-Brazilians, two Japanese, and four Whites) were submitted to genotyping of the alleles that code for the HNA-1 granulocytic antigens using the following set of primers: HNA-1a sense 5′CTCAATGGTACA-CTCAATGGTACAGGGTGCTC3′ (position 128–147) and HNA-1a antisense 5′GGCCTGGCTTGAGATGAGGT3′ (position 227–247); HNA1b sense 5′CTCAATGGTACAGCGTGCTT3′ (position 128–147) and HNA-1b antisense 5′CACTCGTACTCTCCACTGTCGTT3′ (position 277–299). The reaction was carried out with 0.15 µM of each primer, 0.1 mM dNTPs (Applied Biosystem, Foster City, CA), 0.5 U Taq polymerase (Amersham Pharmacia, Piscataway, NJ), and 100 ng of the DNA sample (a total volume of 25 µl). The reaction consisted of 35 cycles of 30 s at 94°C, 30 s at 63°C, 40 s at 72°C, and a final extension at 10 min at 72°C. Amplification with the HNA-1a primers yielded a product of 118 bp, and amplification with the HNA-1b primers yielded a product of 171 bp. The reaction was visualized by 2% agarose gel electrophoresis and ethidium bromide staining. This allele-specific PCR is designed to amplify segments of the *FCGR3B* gene.

Fifty-µl aliquots of the PCR product were purified with the Concert Rapid PCR Purification System kit (Gibco BRL, Gaithersburg, MD). The sequencing reaction was carried out using the ABI Prism Big Dye kit (Applied Biosystems) and the set of sense primers 4NA and 5NA. Additionally, samples with only G base at position 227, C/A bases at position 266, or with variant base composition were sequenciated using primers HNA-1a sense, HNA-1b sense, and HNA-1b antisense. The procedure was carried out using an automatic ABI 377 sequencer (Applied Biosystems). The sequences obtained were edited and aligned using the ABI PRISM 377 DNA Sequencing Analysis program version 3.3 (Applied Biosystem). The alleles corresponding to the granulocytic antigens HNA-1a, HNA-1b, and HNA-1c were identified by analysis of base composition at positions 141, 147, 227, 266, and 277 of the fragment amplified by primers 4NA and 5NA. Combined analysis of base composition at those positions was possible to identify the diverse *FCGR3B* alleles in homozygous and heterozygous state. The distinction of the alleles *FCGR3B*2* and *FCGR3B*3* was done by DNA sequencing of the 253-bp fragment amplified by primers 4NA and 5NA with the primers HNA1b sense and antisense as sequencing primers.

The allele frequencies of the various populations were compared by the Fisher test, with the level of significance set at 5% (InStat version 3.01, GraphPad Software, San Diego, CA).

Genotyping by allele-specific PCR agreed with the results obtained by sequencing in 29 of the 31 samples analyzed (data not presented). As expected, in two variant samples (N1296 and N1375) the results were discordant. Both samples were genotyped by PCR as homozygous for *FCGR3B*2* allele. However, by DNA sequencing both samples were genotyped as heterozygous *FCGR3B*1/FCGR3B*2* at positions 141, 147, 266, and 277. At position 227, the samples were homozygous for a G base indicating that the *FCGR3B*1* was a variant form with A-G substitution at position 227.

Table 1 shows the frequency of the various *FCGR3B* genotypes studied. Among the 60 Indians, the frequency of the homozygous *FCGR3B*1* genotype (HNA-1a/HNA-1a) was 71.7%, ranging from 50% among the Kayapó to 90% among the Awá-Guajá. No *FCGR3B*2* homozygotes (HNA-1b/HNA-1b) were observed in the latter group. Among the 30 individuals of European descent, the frequency of *FCGR3B*1/FCGR3B*2* heterozygotes (HNA-1a/HNA-1b) was 63.4%, with 16.6% *FCGR3B*1* homozygotes (HNA-1a) and 20% *FCGR3B*2* homozygotes (HNA-1b). Among the Japanese individuals, the frequency of *FCGR3B*1/FCGR3B*2* heterozygotes (HNA-1a/HNA-1b) was 51.7%, with only 6.9% *FCGR3B*2* homozygotes (HNA-1b) and 41.1% *FCGR3B*1* homozygotes (HNA-1a). The Afro-Brazilian population studied presented the highest genotypic variability, with 40% of the 30 individuals studied being *FCGR3B*1* homozygotes (HNA-1a) and 13.4% *FCGR3B*2* homozygotes (HNA-1b). Fourteen Blacks were heterozygotes, 30% of them being *FCGR3B*1/FCGR3B*2* (HNA-1a/HNA-1b), 10% *FCGR3B*1/FCGR3B*3* (HNA-1a/HNA-1c), and 6.6% *FCGR3B*2/FCGR3B*3* (HNA-1b/HNA-1c).

Table 1 shows the frequency of *FCGR3B* alleles in the populations studied. The frequency of the *FCGR3B*1* allele was 0.858 among the Amerindians, corresponding to the highest frequency among the populations studied ($P < 0.002$). The frequency of the *FCGR3B*1* allele was 0.483 among Whites, being lower than the frequency detected among Afro-Brazilians (0.600), Japanese (0.672), and Amerindians (0.858) ($P < 0.01$). The frequency of the *FCGR3B*1* allele among Afro-Brazilians (0.600) did not differ significantly from that observed among Japanese (0.672) ($P = 0.378$). The *FCGR3B*3* allele was detected only in the Afro-Brazilian population, at a frequency of 0.083. With the methodology used, it was not possible to determine the occurrence of two or three genes in relation to the *FCGR3B*3* allele.

Two variant haplotypes were detected by DNA sequencing among the 118 Amerindians and the 30 Afro-Brazilians and are described in Table 2. Samples W50 and AW48 were heterozygous, presenting a typical *FCGR3B*1* haplotype and a variant *FCGR3B*2* haplotype with a guanine at position 277 (*FCGR3B*2 A277G*). Samples KBK78 and KBK91 were homozygous and samples N1296 and N1375 were heterozygous for a variant *FCGR3B*1* haplotype with a guanine at position 227 (*FCGR3B*1 A227G*). The confirmation that this variant was located in *FCGR3B*1* gene and not in *FCGR3A* gene was done by a combination of PCR-allele-specific and four cycles of DNA sequencing using primers 4NA, 5NA, and HNA-1a sense, that reveals a homozygous G at position 227. If the variation was located in *FCGR3A* gene, we must have bases G/A at position 227 which was not the case.

FCGR3B genotype and allele frequency in Brazilian White, Afro-Brazilian, Japanese, and Amerindian populations

		FCGR3B genotype *n* (%)					Allele*		
Population	*n*	1/1	1/2	2/2	1/3	2/3	*FCGR3B1*	*FCGR3B2*	*FCGR3B3*
Euro-Brazilian	30	5 (16.6)	19 (63.4)	6 (20)	–	–	0.48 (29)	0.51 (31)	–
Afro-Brazilian	30	12 (40)	9 (30)	4 (13.4)	3 (10)	2 (6.6)	0.60 (36)	0.31 (19)	0.08 (5)
Japanese	29	12 (41.1)	15 (51.7)	2 (6.9)	–	–	0.66 (39)	0.32 (19)	–
Indians	60	43 (71.7)	17 (28.3)	–	–	–	0.85 (103)	0.14 (17)	–
Yanomama	9	8 (89)	1 (11)	–	–	–	0.94 (17)	0.06 (1)	
Arara	4	3 (75)	1 (25)	–	–	–	0.88 (7)	0.12 (1)	
Kayapó	16	8 (50)	8 (50)	–	–	–	0.75 (24)	0.25 (8)	
Wayana-Apalai	10	8 (80)	2 (20)	–	–	–	0.90 (18)	0.10 (2)	
Wayampi	11	7 (64)	4 (36)	–	–	–	0.83 (18)	0.18 (4)	
Awá-Guajá	10	9 (90)	1 (10)	–	–	–	0.95 (19)	0.05 (1)	

*Frequency (number of alleles observed).

Table 1

Base composition at positions 141, 147, 227, 266, and 277 of six FCGR3B gene variants observed among Afro-Brazilian and Amerindian populations

	Polymorphic nucleotide position					
Donor Id	141	147	227	266	277	FCGR3B allele
Prototypes	G	C	A	C	G	*FCGR3B*1*
	C	T	G	C	A	*FCGR3B*2*
	C	T	G	A	A	*FCGR3B*3*
W5O and Aw 48	G	C	A	C	G	*FCGR3B*1*
	C	T	G	C	**G**	*FCGR3B*2 A277G*
KBK 78 and KBK 91	G	C	**G**	C	G	*FCCR3B*1 A227G*
	G	C	**G**	C	G	*FCCR3B*1 A227G*
N 1296 and N 1375	G	C	**G**	C	G	*FCGR3B*1 A227G*
	C	T	G	C	A	*FCGR3B*2*

Table 2

In the present study, using DNA sequencing we determined the gene frequency of alleles of the *FCGR3B* gene in 149 individuals belonging to four distinct Brazilian populations, i.e., Amerindians, Whites, Afro-Brazilians, and Japanese. We describe for the first time a variant A227G form of the *FCGR3B*1* allele detected in two Amerindians and in two Blacks. We detected a previously described variant A277G form of the *FCGR3B*2* allele in homozygosis in two Amerindians.

The frequency of the *FCGR3B*1* allele among Amerindians was 0.858, representing the highest gene frequency described thus far for this allele in population studies. This frequency was higher than that observed in another group of Brazilian Amerindians (0.670. $P < 0.0001$) and in North-American Indians (0.551, $P < 0.0001$) (12, 16). The explanation for this discrepancy may reside in the fact that the populations of Brazilian Amerindians present a higher- or lower-population isolation. The tribes studied, in particular, located in the Brazilian Amazon Region, were geographically isolated, with little evidence of gene exchange with other populations, and significant variations of gene frequencies among different tribes, as also shown by the study of other genes (13–15). The frequency of the *FCGR3B*1* gene among Amerindians was also higher than among Japanese living in Brazil and Japanese living in Japan (0.660, $P = 0.005$) and among Chinese (0.681, $P < 0.0001$) (17, 18). These three populations have a common Asian origin that might explain the high frequency observed for this allele. The higher frequency among Amerindians may be due to the genetic drift during or after migration to America. As these genes are directly involved with the response to environment, especially defense to infection, a selective pressure cannot be excluded.

Among Amerindians, we observed the occurrence of two variant forms of the *FCGR3B* allele in four individuals. The *FCGR3B*2 A277G* allele was observed in heterozygosis with the *FCGR3B*1* allele in two individuals belonging to different tribes (Awá-Guajá and Wayana Apalai). This allele had been previously described in North-American Blacks (9). The *FCGR3B*1 A227G* allele is described here for the first time and was detected in homozygosis in two individuals belonging to the Kayapó tribe. The null *FCGR3B* and *FCGR3B*3* alleles were not observed among Amerindians.

Among the Afro-Brazilians studied, the *FCGR3B*1* allele occurred at a frequency of 0.600, which was significantly higher than the frequency observed in Afro-North Americans (0.310, $P < 0.0001$) (12). This observation may be due to the fact that Afro-Brazilians and Afro-Americans originate from different African regions (13, 19). The frequency of the *FCGR3B*1* allele among Afro-Brazilians did not differ from that observed in Brazilian patients with sickle-cell anemia (16), a result explained by the fact that sickle-cell anemia is more prevalent among African individuals or individuals of African descent.

The *FCGR3B*3* allele was only detected in the population of Afro-Brazilians, where it was present at a frequency of 0.083. This frequency was significantly lower than that observed among Afro-Americans (0.207; $P < 0.05$), Ugandans (0.166, $P < 0.0001$), and South Africans (0.191, $P = 0.0007$) (9, 10, 17). In two of the five cases in which this allele was observed, the allele occurred in association with the *FCGR3B*2* allele, and in the other three it occurred in association with the *FCGR3B*1* allele. These data do not permit us to state that this allele presents linkage with any of these alleles, as previously observed (20) The methodology used in the present study did not permit the detection of *FCGR3B* gene duplication, as reported in other studies (21). As proposed by Bux et al., this observation could be explained by the fact that the point mutation that gave origin to the *FCGR3B*3* allele first occurred in Africa and the duplication of the *FCGR3B* gene may have occurred later, explaining both the identification of individuals with two and three *FCGR3B* genes and the presence or absence of linkage (22–24). Further studies are needed to clarify this point.

The frequency of the *FCGR3B*1* allele observed among the Japanese population of Brazil (0.660) did not differ from the frequency observed in other studies conducted in Japan (0.651) and in the Japanese population of the United States (0.630) (9, 17). No variant forms of the *FCGR3B* gene were observed in this population, in contrast to the study by Matsuo et al. (9), which demonstrated the occurrence of variant forms among Japanese people living in the United States (9).

In the White population, the frequency of the *FCGR3B*1* allele was 0.480 a value that did not differ from the frequency observed in similar populations from other countries (11, 12, 20).

The variant forms of the *FCGR3B* allele observed in the present study and others are probably the results of crossover between the *FCGR3B*1* and *FCGR3B*2* alleles. Other possibilities are the

presence of a larger number of gene loci and the occurrence of somatic hypermutation (10). Further studies are needed to clarify these questions and others concerning the functional evaluation of the receptors coded by these variant alleles.

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