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Brief Communications

Ethnic Differences in the Distribution of Interleukin-6 Polymorphisms Among Three Brazilian Ethnic Groups

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Abstract Polymorphisms in the interleukin-6 promoter region have been associated with diseases. In this study we investigated the *−634G/C* and *−174G/C* IL-6 promoter polymorphisms in three Brazilian ethnic groups. We verified that the allele frequencies of the two polymorphisms and haplotype frequencies varied significantly between the populations.

Cytokine gene variants located in the promoter or regulatory regions may affect cytokine production and change the intensity and/or quality of the immune response (Nishimura et al. 2002; Meenagh et al. 2002).

Interleukin 6 (IL-6), a pleiotropic cytokine synthesized by many cell types, is not constitutively expressed; rather, it is produced in response to many inflammatory stimuli (Ershler and Keller 2000). The IL-6 gene is located on chromosome 7p21, and variants in its promoter have been analyzed. The *−174G/C* variant lies immediately upstream from multiresponsive element (MRE) and is associated with IL-6 gene transcription (Fishman et al. 1998). This polymorphism has been studied extensively and is associated with juvenile rheumatoid arthritis, Alzheimer's disease, hyperandrogenism, insulin sensitivity, susceptibility to type I diabetes mellitus, and cardiovascular disease (Fishman et al. 1998; Jahromi et al. 2000; Jenny et al. 2002; Villuendas et al. 2002; Licastro et al. 2003; Kubaszek et al. 2003).

Other common mutations in the IL-6 promoter region also might be associated with disease. The *−634G/C* polymorphism was previously shown to be associated with progression of diabetic nephropathy, bone mineral density, and susceptibility to HTLV-1 associated myelopathy (Ota et al. 2001; Kitamura et al. 2002; Nishimura et al. 2002).

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The *−174C* promoter allele frequency is highly heterogeneous among different populations (Fishman et al. 1998; Cox et al. 2001; Hoffmann et al. 2002), but the *−634G/C* variant has been studied only in the Japanese population (Nishimura et al. 2002; Kitamura et al. 2002; Yamada et al. 2003).

Brazil is a nation of several ethnic groups and cultural characteristics. The admixture of Portuguese, indigenous tribes, Africans, and the resulting interethnic crosses between these peoples have created an extremely rich ethnic and cultural diversity (Alves-Silva et al. 2000). Besides that, a variety of immigrants have also contributed to the diverse ethnic, cultural, and genetic composition of Brazil (Callegari-Jacques and Salzano 1999; Alves-Silva et al. 2000). However, the contributions of each continental group vary among different regions of Brazil (Dornelles et al. 1999).

Because the investigation of *−634G/C* and *−174G/C* IL-6 polymorphisms in populations with different ethnic backgrounds can contribute to a better understanding of the complex association between IL-6 promoter variability and disease susceptibility, we analyzed the distribution of these two polymorphisms and their resulting haplotypes in three Brazilian populations with different ethnic backgrounds.

Materials and Methods

We examined 293 genomic DNA samples from distinct populations of Brazil that had been collected between 1997 and 2001 as part of previous studies: 100 samples from the general population of Salvador (Dourado et al. 2003), approximately 80% of which have mixed Portuguese and African ancestry (Alcantara et al. 2003), representing the Brazilian northeast region; 94 samples from the southern region, collected from German descendants at the Joinville blood center, Santa Catarina State (Grimaldi et al. 2002); and 99 samples from the Tiriyó tribe, who live in northern Brazil along the border of Suriname (Shindo et al. 2002).

Genomic DNA was extracted from peripheral blood mononuclear cells using a proteinase K treatment followed by a phenol-chloroform method. The analysis of the *−634G/C* IL-6 polymorphism was done through PCR amplification followed by restriction fragment length polymorphism (RFLP) using primers and PCR conditions described by Nishimura et al. (2002). To determine *−174G/C* IL-6 polymorphism, an SNP assay with real-time PCR was done using the following primers: 5′-GACGACCTAAGCTGCACTTTTC and 5′-GGGCTGATTGGAAACCTTATTAAGATTG-3′. In the same reaction we used fluorescent probes: 5′-CCTTTAGCAT(C)GCAAGAC-3′ for the *C* allele and 5′-CTTTAGCAT(G)GCAAGAC-3′ for the *G* allele, according to the manufacturer's instructions (Applied Biosystems).

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy-Weinberg expectations were tested using Arlequin, version 2.000 (Schneider et al. 2000). The heterogeneity of polymorphism

Table 1. Genotype and Allele Frequencies (%) of Interleukin-6 Promoter Polymorphism at Positions *−634G/C* and *−174G/C* in Three Brazilian Populations

	Genotype			*Allele*	
	G/G (N and %)	*G/C (N and %)*	*C/C (N and %)*	*G (N and %)*	*C (N and %)*
−634					
Salvador	68 (68.0)	30 (30.0)	2 (2.0)	166 (83.0)[a]	34 (17.0)[a]
Tiriyó	18 (18.2)	52 (52.5)	29 (29.3)	88 (44.4)[a]	110 (55.6)[a]
Joinville	87 (92.6)	7 (7.4)	–	181 (96.3)[a]	7 (3.7)[a]
−174					
Salvador	71 (71.0)	25 (25.0)	4 (4.0)	167 (83.5)[b]	29 (14.5)[b]
Tiriyó	94 (94.9)	5 (5.1)	–	193 (97.5)[b]	5 (2.5)[b]
Joinville	43 (45.75)	33 (35.1)	18 (19.15)	119 (63.3)[b]	69 (36.7)[b]

Adjusted chi-square residues: $p < 0.01$.
a. $\chi^2 = 147.648$, 2 df, $p < 0.001$ (allele frequency).
b. $\chi^2 = 76.100$, 2 df, $p < 0.001$ (allele frequency).

frequencies among populations were tested using a chi-square test followed by an adjusted chi-square residual analysis or with Fisher's exact test using PEPI, version 4.0 (Abramson and Gahlinger 2001). A p value $\leqslant 0.05$ was considered statistically significant, and Bonferroni's correction for multiple comparisons was used.

Results and Discussion

The distribution of observed *−634G/C* and *−174G/C* IL6 genotype and allele frequencies is shown in Table 1. Genotype frequencies from both sites did not differ significantly from those expected under Hardy-Weinberg equilibrium after applying the Bonferroni correction. There was significant linkage disequilibrium between *−634G/C* and *−174G/C* polymorphisms in both Salvador ($D' = -1$, $p = 0.00461$) and Joinville ($D' = -1$, $p = 0.02140$) but not in the Tiriyó tribe ($p = 0.35875$). The allele frequencies of the *−634G/C* ($p < 0.001$) and *−174G/C* ($p < 0.001$) polymorphisms differed among the three regional Brazilian populations. A similarly heterogeneous pattern was observed in the *−174G/C* polymorphism frequencies (Table 1).

Table 2 shows the estimated two-site IL-6 promoter haplotype frequencies for the three populations. The haplotype frequencies varied significantly ($p < 0.0001$).

The *−174C* allele frequency is highly heterogeneous among different populations (Table 3). The higher frequency of the *−174C* allele in the German descendants from Joinville is consistent with those observed previously in European and European-derived populations (Table 3). The frequency of the *−174C* allele in the Salvador sample, although higher than those previously observed in

Table 2. Haplotype Frequencies (%) at the Interleukin-6 Promoter Region in Three Different Populations from Brazil (*−634G/C* and *−174G/C* Combination)

	Salvador (n = 200)	*Tiriyó (n = 198)*	*Joinville (n = 188)*
−634G/−174G	66.5	42.8	59.1
−634G/−174C	16.5	1.6	37.2
−634C/−174G	17.0	54.8	3.7
−634C/−174C	–	0.8	–

n = Chromosome number.
Salvador × Tiriyó: Fisher's exact test, $p < 0.0001$.
Salvador × Joinville: Fisher's exact test, $p < 0.0001$.
Tiriyó × Joinville: Fisher's exact test, $p < 0.0001$.

Africans and African Americans, is close to frequencies previously reported (Table 3). This is consistent with historical records that show the importance of the African contribution to the ethnic admixture with European descendents in Salvador (Alcantara et al. 2003). The lowest *−174C* allele frequency was found in the Amerindian Tiriyó tribe. This frequency is in the range of frequencies observed in Asian populations (Table 3) and is consistent with the theorized Asian origin of the autochthonous Amerindians, confirming previous studies on several other polymorphic systems (Ribeiro et al. 2003; Tokunaga et al. 2001).

Few studies have investigated the *−634C/G* IL-6 gene polymorphism, and all were carried out in Asian populations. The *−634G* allele frequencies in three previous studies of Japanese varied from 15.5% to 22.2% (Table 3). A great heterogeneity was detected in the frequencies of *−634G/C* IL-6 promoter polymorphism among Brazilian samples (Table 1). The mainly African-derived population from Salvador and the German descendants from Joinville showed higher *−634G* allele frequencies. This is the first report of the *−634C/G* variant in these ethnic groups, and further studies on the variability of this frequency in the European and African descendants are needed.

A previous study in Japan showed an association between the *−643G* variant and the development of human T-cell leukemia/lymphoma virus type 1

Table 3. Interleukin-6 *−174G/C* Allele Frequencies (%) in Three Major Ethnic Groups

IL-6 174 Allele	*African/African Amerindians*	*Asian/Indians*	*Whites*
G	89.7–90.7[a,b]	95.5–100[b–e]	61.7[b]
C	9.3–10.3[a,b]	0.2–4.5[b–e]	38.3[b]

a. Cox et al. (2001).
b. Hoffmann et al. (2002).
c. Hayakawa et al. (2002).
d. Zhai et al. (2001).
e. Fishman et al. (1998).

(HTLV-1) associated myelopathy (Nishimura et al. 2002). Salvador has the highest HTLV-1 prevalence in Brazil (1.35–1.76%) (Galvão-Castro et al. 1997; Dourado et al. 2003). Because the *−634G* variant is also common in Salvador (Table 1), it is important to investigate the impact of this polymorphism on the development of HTLV-1 associated myelopathy in our population. Indeed, our preliminary results demonstrated that the *−634G* allele was more frequent in the asymptomatic carriers than in patients with TSP/HAM (tropical spastic paraparesis/human T-cell leukemia virus type 1 associated myelopathy), suggesting that this allele can have a protective effect against the development of TSP/HAM (Gadelha et al. 2004).

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