

Factor VIII gene polymorphisms in Amerindians from the Brazilian Amazon region

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ABSTRACT

We studied factor VIII gene polymorphisms in 95 Amerindians from five Amazon tribes: Wayampí, Wayana-Apaláí, Kayapó, Arára and Yanomámi. Six polymorphic sites were analyzed: *BclI* (intron 18), *HindIII* (intron 19), *XbaI* A (intron 22), *BglI* (intron 25), and two extragenic *XbaI* sites (*XbaI* B and *XbaI* C, located at 5' of the gene). The allele frequencies distribution in Amerindians was similar to that observed for Asian and Caucasian populations, but clearly differed from the pattern seen in Blacks. Allele frequencies for the *BclI*, *HindIII* and *XbaI* A polymorphisms were not significantly different among the five tribes, whereas *BglI*, *XbaI* B and *XbaI* C sites showed a heterogeneous distribution. These findings indicate heterogeneity among the Amerindian tribes, in contrast with the lack of diversity observed for the β -globin polymorphisms, but in agreement with our previous results for α -globin and VNTR analysis for the same tribes. Our results demonstrate genetic diversity among different Amerindian tribes. In addition, our study indicate that analysis of the factor VIII gene polymorphisms may represent a useful tool for population genetics studies.

INTRODUCTION

Analysis of DNA polymorphisms has been used as a powerful tool for investigation of evolutionary relationships between human populations. At present, a large number of polymorphisms can be used for that purpose, those presenting diverse patterns of allelic distribution according to the ethnic origin of the population under investigation being particularly useful. Factor VIII-associated polymorphisms represent an example of such a group of polymorphisms, because their analyses have produced different results depending on the racial group studied (Peake *et al.*, 1993).

Despite many previous studies, a number of controversies still persist in relation to Brazilian Amerindians, especially regarding the peopling (single

or multiple origin) of the Americas and the extent of the genetic diversity among the numerous Indian tribes (Guerreiro *et al.*, 1994). To date, few studies based on DNA analysis have been conducted on Amerindian populations (Guerreiro *et al.*, 1994; Franco *et al.*, 1994; Heidrich *et al.*, 1995; Zago *et al.*, 1995), and there are no data describing factor VIII gene polymorphisms in this racial group.

In the present article we describe the analysis of six polymorphic restriction sites associated with the factor VIII gene in five Amazonian Indian tribes and compare the findings with those reported for other human populations.

SUBJECTS AND METHODS

Subjects

Ninety-five Indians (48 males and 47 females) from five tribes from the Brazilian Amazon region were

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studied: 17 Wayampí, 22 Wayana-Apalaí, 21 Kayapó, 15 Arára and 20 Yanomámi; all the subjects included are apparently unrelated based on pedigree data. The five tribes are located in the Brazilian Amazon region (for exact locations see Guerreiro *et al.*, 1994). The Kayapó Indians speak a Jê language, the Wayampí belong to the Tupí-Guaraní linguistic stock, and the Wayana-Apalaí and Arára speak Karíb languages. The Yanomámi speak Ninam, which is one of the four closely related languages that constitute the Yanomámi linguistic family (Rodrigues, 1986). The polymorphic sites linked to the factor VIII gene were investigated in all 95 Amerindians.

DNA analysis

Genomic DNA samples were obtained from blood leucocytes by phenol-chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989). Six polymorphic sites linked to the factor VIII gene were studied: *BclI* (intron 18), *HindIII* (intron 19), *XbaI* A (intron 22), *BglII* (intron 25), and two *XbaI* extragenic sites (*XbaI* B and *XbaI* C, located at about 500 kb from the 5' end of the gene) (Chan *et al.*, 1989; Taylor *et al.*, 1989; Patterson *et al.*, 1989; Wehnert *et al.*, 1990; Bowen and Bloom, 1993). The *BclI* and *HindIII* sites were analyzed by the polymerase chain reaction. The other sites were analyzed by the Southern blotting/hybridization method.

Polymerase chain reaction (PCR)

The *BclI* and *HindIII* polymorphic sites were investigated using the primers described previously (Silva Jr. and Figueiredo, 1994). PCR experiments were performed in 25 µl total reaction volume containing 200 ng (*BclI*) or 100 ng (*HindIII*) of genomic DNA, 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM MgCl₂, 0.01% BSA, 200 µM of each dNTP, 0.25 µM (*BclI*) or 0.13 µM (*HindIII*) of each primer, and 1 U of Taq DNA polymerase; 35 cycles of amplification were carried out (94°C for 30 sec, 55°C for 20 sec, and 72°C for 60 sec). After PCR, the products were digested with the appropriate enzyme in excess and the fragments were separated on 6% polyacrylamide gel (*BclI* site) or 3% agarose gel (*HindIII* site). Negative

and positive controls for each polymorphism were included in all experiments.

Southern blotting/hybridization assay

Ten µg of DNA was digested with the appropriate enzymes, separated on 1% agarose gel, and transferred to nylon membranes by the capillary method (Sambrook *et al.*, 1989). The filters were hybridized to specific probes at 42°C for 16-48 h, washed at 65°C for 60 min with 0.1 x SSC, 0.1% SDS, and exposed to X-ray film (X-Omat AR, Kodak) with intensifying screens at -70°C from one to seven days. The following probes were used: p1.8 for *BglII* polymorphism (Antonarakis *et al.*, 1985) and a 1.1-Kb *EcoRI/SstI* fragment from the genomic probe p482.6 for *XbaI* polymorphisms (Wion *et al.*, 1986). For the latter study, DNA samples were double-digested with *XbaI* and *KpnI*. The probes were labelled with [α -³²P]dCTP using Random Primed DNA labelling (Sambrook *et al.*, 1989).

RESULTS

Allele frequencies for six factor VIII gene-associated polymorphisms were determined in the 95 Amerindians, and the results are summarized in Table I. The heterozygosity for each polymorphism among the five tribes is shown in the same table. Distribution of the allele frequencies for the *BclI*, *HindIII* and *XbaI* A polymorphisms did not differ significantly

Table I - Factor VIII gene polymorphisms among five Indian tribes from the Brazilian Amazon region. The frequencies for the presence of the polymorphic site (p), the number of chromosomes analyzed (N), and heterozygosity (observed/expected) for each polymorphism are presented.

Tribe	Polymorphism (p)					
	<i>BclI</i>	<i>HindIII</i>	<i>XbaI</i> A	<i>BglII</i>	<i>XbaI</i> B	<i>XbaI</i> C
Arára	0.55 (N = 20)	0.45 (N = 20)	0.57 (N = 23)	1.0 (N = 23)	0 (N = 23)	0 (N = 23)
Wayana-Apalaí	0.60 (N = 30)	0.44 (N = 32)	0.47 (N = 34)	0.82 (N = 28)	0 (N = 34)	0 (N = 34)
Yanomámi	0.74 (N = 31)	0.26 (N = 31)	0.71 (N = 31)	0.84 (N = 31)	0.10 (N = 31)	0.10 (N = 31)
Kayapó	0.61 (N = 31)	0.39 (N = 31)	0.61 (N = 31)	1.0 (N = 31)	0.26 (N = 31)	0.19 (N = 31)
Wayampí	0.52 (N = 23)	0.50 (N = 22)	0.55 (N = 22)	1.0 (N = 22)	0 (N = 22)	0 (N = 22)
Total*	0.70 (N = 135)	0.41 (N = 136)	0.58 (N = 141)	0.92 (N = 135)	0.07 (N = 141)	0.06 (N = 141)
Heterozygosity*	0.26/0.47	0.34/0.47	0.40/0.48	0.07/0.14	0.13/0.14	0.13/0.13

*Combined results for all five tribes.

among the five tribes. On the other hand, heterogeneity was observed for the allele frequencies distribution for the sites *Bgl*I ($\chi^2 = 13.9$; $P < 0.01$), *Xba*I B ($\chi^2 = 20.8$; $P < 0.005$), and *Xba*I C ($\chi^2 = 14.6$; $P < 0.01$). The Wayampí, Kayapó, and Arára showed monomorphism for the positive allele of *Bgl*I, in contrast to the findings in the Wayana-Apalaí and Yanomámi. The presence of the extragenic polymorphic sites *Xba*I B and *Xba*I C was restricted to the Yanomámi and Kayapó. An example of analysis of the *Xba*I sites is illustrated in Figure 1; the 5.2- and 5.4-kb bands indicate the presence of the polymorphic sites *Xba*I B and *Xba*I C, respectively. In addition to population studies, the analysis of these extragenic *Xba*I polymorphisms has been used occasionally for hemophilia A carrier detection (Figueiredo *et al.*, 1993).

DISCUSSION

The allele frequencies pattern observed for the Amerindians was similar to that found among Asian and Caucasian populations, but differed substantially from that observed for Black populations (Table II). At present, comparison of the results obtained for the extragenic *Xba*I sites is not possible since there is no data reporting their analyses in other racial groups.

Our findings are in agreement with those obtained previously by the analysis of other gene systems in the same population, which demonstrate genetic similarities between South American Indians and Asian and Polynesian populations (Guerreiro *et al.*, 1994; Zago *et al.*, 1995). Therefore, our results may be regarded as additional evidence for the hypothesis of Asiatic origin of the native populations of America.

The heterogeneity for Indian subpopulations suggested by our study is in contrast to our study of β -globin gene polymorphisms (Guerreiro *et al.*, 1994), in which we demonstrated a high level of homogeneity of β -globin haplotypes among the same Indian tribes. Estimates of intertribal heterogeneity can differ depending on the polymorphisms studied. Whereas homogeneity was observed by the study of β -globin haplotypes, intertribal genetic diversity was recently demonstrated by the study of six VNTRs (Zago *et al.*, in press) and α -globin haplotype analysis (Zago *et al.*, 1995). These conflicting results may be explained by a combination of genetic mechanisms: founder effect, genetic drift, gene flow, and consanguinity, acting on small isolated groups with a previous complex history of divergence-fusion events (Zago *et al.*, 1995). Taken together, these data support the idea that the study of a larger number of polymorphisms and Indian tribes is

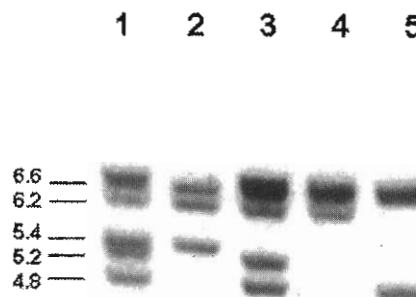


Figure 1 - Analysis of *Xba*I sites. The alleles for the intragenic site *Xba*I A are identified by the 6.2-kb (negative allele) and 4.8-kb (positive allele) bands. The alleles for the extragenic sites *Xba*I B and *Xba*I C are identified by the 6.6-kb band (negative alleles) and 5.2- and 5.4-kb bands (positive alleles for the sites *Xba*I B and *Xba*I C, respectively). *Lane 1*: Female, heterozygous for sites *Xba*I A, B, and C; *lane 2*: male, showing the negative allele for sites *Xba*I A and B, and the positive allele for site *Xba*I C; *lane 3*: female, heterozygous for sites *Xba*I A and B, and homozygous for the negative allele of site *Xba*I C; *lane 4*: male, showing the negative allele for all *Xba*I sites; *lane 5*: male, showing the positive allele for site *Xba*I A and negative alleles for sites *Xba*I B and C.

Table II - Frequencies of the presence of four polymorphic sites linked to the factor VIII gene in different ethnic groups.

Polymorphism	Population			
	Amerindians ¹	Brazilian Blacks ²	Caucasians ³	Asians ³
<i>Bcl</i> I	0.70	0.22	0.73	0.69
<i>Hind</i> III	0.41	0.80	0.26	0.29
<i>Xba</i> I A	0.58	0.05	0.56	ND
<i>Bgl</i> I	0.92	0.80	0.85	0.94

ND, Not determined; ¹present work, combined results for all five tribes; ²Silva Jr. and Figueiredo (1994); ³Peake *et al.* (1993).

necessary to obtain accurate information about the genetic diversity within the Amerindian population.

In conclusion, our findings demonstrate heterogeneity among Amerindian tribes. Additionally, our study suggests that analysis of factor VIII gene-associated polymorphisms is an important tool for population and human evolution studies.

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RESUMO

Determinamos as freqüências alélicas de polimorfismos de restrição ligados ao gene do fator VIII da coagulação em 95 indígenas provenientes de cinco tribos da Amazônia: Wayampí, Wayana-Apalaí, Kayapó, Arára e Yanomámi. Seis sítios polimórficos foram analisados: *BclI* (intron 18), *HindIII* (intron 19), *XbaI* A (intron 22), *BglI* (intron 25) e dois sítios extragênicos *XbaI* (*XbaI* B e *XbaI* C, localizados a 5' do gene). A distribuição dos polimorfismos do gene do fator VIII em Ameríndios foi similar à observada em populações asiáticas e caucasóides, mas diferiu claramente do padrão observado em negros. A distribuição das freqüências alélicas dos polimorfismos intragênicos *BclI*, *HindIII* e *XbaI* não diferiu significativamente entre as cinco tribos. Por outro lado, houve heterogeneidade das freqüências alélicas para os sítios *BglI* e *XbaI* B e *XbaI* C, indicando uma possível heterogeneidade entre as tribos ameríndias. Esses resultados se contrapõem à ausência de diversidade observada com a análise dos polimorfismos dos genes das globinas β -símbles, mas concordam com nossos resultados recentes obtidos através da análise de haplótipos de genes da globina alfa e de 6 VNTRs nas mesmas populações. Dessa forma, nossos resultados representam uma evidência adicional da heterogeneidade entre diferentes tribos ameríndias e reforçam a necessidade de se estudar um maior número de sistemas gênicos para uma melhor caracterização das interações genéticas entre essas subpopulações. Demonstra-se, também, que os polimorfismos associados ao gene do fator VIII podem representar uma ferramenta útil em estudos de genética populacional.

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