



## Genetic relationships among native americans based on $\beta$ -globin gene cluster haplotype frequencies

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### Abstract

The distribution of  $\beta$ -globin gene haplotypes was studied in 209 Amerindians from eight tribes of the Brazilian Amazon: Asurini from Xingú, Awá-Guajá, Parakanã, Urubú-Kaapór, Zoé, Kayapó (Xikrin from the Bacajá village), Katuena, and Tiriyo. Nine different haplotypes were found, two of which (n. 11 and 13) had not been previously identified in Brazilian indigenous populations. Haplotype 2 (+ - - -) was the most common in all groups studied, with frequencies varying from 70% to 100%, followed by haplotype 6 (- + + -), with frequencies between 7% and 18%. The frequency distribution of the  $\beta$ -globin gene haplotypes in the eighteen Brazilian Amerindian populations studied to date is characterized by a reduced number of haplotypes (average of 3.5) and low levels of heterozygosity and intrapopulational differentiation, with a single clearly predominant haplotype in most tribes (haplotype 2). The Parakanã, Urubú-Kaapór, Tiriyo and Xavante tribes constitute exceptions, presenting at least four haplotypes with relatively high frequencies. The closest genetic relationships were observed between the Brazilian and the Colombian Amerindians (Wayuu, Kamsa and Inga), and, to a lesser extent, with the Huichol of Mexico. North-American Amerindians are more differentiated and clearly separated from all other tribes, except the Xavante, from Brazil, and the Mapuche, from Argentina. A restricted pool of ancestral haplotypes may explain the low diversity observed among most present-day Brazilian and Colombian Amerindian groups, while interethnic admixture could be the most important factor to explain the high number of haplotypes and high levels of diversity observed in some South-American and most North-American tribes.

*Key words:* DNA polymorphisms, Brazilian Amerindians, genetic diversity.

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### Introduction

The molecular polymorphism of the  $\beta$ -globin gene cluster was the first nuclear DNA segment widely studied for the analysis of evolutionary relationships among human populations (Wainscoat *et al.*, 1986). The distribution of haplotypes associated with the  $\beta$ A gene is now known for a large number of populations. The data indicate that Africans are the most divergent group, and that the first split separated an African group from an Eurasian one (Wainscoat *et al.*, 1986; Oehme *et al.*, 1985; Antonarakis *et al.*, 1985; Maggio *et al.*, 1986; Kulozik *et al.*, 1986; Ramsay and Jenkins, 1987; Hundrieser *et al.*, 1988a,b;

Yongvanit *et al.*, 1989; Chen *et al.*, 1990; Long *et al.*, 1990; Trent *et al.*, 1990; Shimizu, 1987; Shimizu *et al.*, 1989; Varawalla *et al.*, 1992; Penaloza *et al.*, 1995; Hewitt *et al.*, 1996; Castro-de-Guerra *et al.*, 1997; Fucharoen *et al.*, 1997; Kaufman *et al.*, 1998; Villalobos-Arámbula *et al.*, 2000). The distribution of  $\beta$ A haplotypes in Brazilian Amerindian populations was investigated in ten tribes of the Amazon region by Guerreiro *et al.* (1992, 1994) and Bevilacqua *et al.* (1995), who obtained similar results. Haplotypes 2 and 6 were the most common, and heterozygosity was reduced, as compared to Europeans and Africans. The results also showed that Brazilian Amerindians are closely related to Asians, Polynesians and Micronesians. However, genetic studies of Brazilian Amerindians based on tandemly repeated minisatellite loci have shown that, although heterozygosity and the mean number of alleles are low, there is a marked heterogeneity

among populations, with distinct patterns of allele distribution in different tribes. This suggests that the study of a small number of tribes may not adequately sample the whole range of genetic variability in Brazilian Amerindians (Zago *et al.*, 1996). Here, we report on the distribution of the  $\beta$ -globin gene haplotypes in eight new Amerindian tribes from the Amazon region of Brazil, and compare our results with those reported previously for Native American and other human populations.

## Subjects and Methods

The study was performed on apparently unrelated subjects from eight Amerindian tribes from the Brazilian Amazon region: Asurini of the Xingú (3°43' S; 52°27' W), Awá-Guajá (3°30' S; 46°40' W), Parakanã (5°45' S; 51°52' W), Urubú-Kaapór (2°48' S; 46°10' W), Zo'é (0°18' N; 55°18' W), Kayapó (Xikrin of Bacajá village; 3°46' S; 51°35' W), Katuena (0°40' S; 57°30' W), and Tiryó (3°30' S; 53°21' W).

Blood samples were collected using the anticoagulant EDTA, and genomic DNA was isolated from whole blood by phenol-chloroform extraction and ethanol precipitation (Old and Higgs, 1983).  $\beta$ -globin gene haplotypes were identified by analyzing the following polymorphic restriction sites: 1. HincII 5'; 2. HindIII G; 3. HindIII A; 4.

HincII- $\beta$ ; 5. HincII-3' $\beta$ . Typing was done by PCR amplification, followed by restriction digestion and agarose gel electrophoresis, according to Guerreiro *et al.* (1992) for site HincII 5', and to Sutton *et al.* (1989) for the remaining sites. Haplotype identification was made using the likelihood method described by Excoffier and Slatkin (1995). Haplotype diversity, the gene differentiation coefficient (Nei, 1973, 1978; Livshits and Nei, 1990), and genetic distances (DA distance, as described by Nei *et al.*, 1983) between Brazilian Amerindians and other populations were estimated using the DISPAN program (Ota, 1993). A neighbor-joining tree (Saitou and Nei, 1987) was obtained from DA genetic distances using the same program. Comparisons of the haplotype distribution in different populations were made with Pearson's Chi-square statistic using the Biosys-1 program (Swofford and Selander, 1981).

## Results

Nine different haplotypes were found, two of which (n. 11 and 13) had not been previously identified in Brazilian indigenous populations (Table 1). The number of identified haplotypes per tribe ranged from one in the Awá-Guajá to eight in the Urubú-Kaapór. Haplotype 2 (+ - - -) was the most common in all groups studied, with frequencies varying from 70% to 100%, followed by haplo-

**Table 1** -  $\beta$ -globin gene haplotypes in Brazilian Amerindians and other native Americans.

Populations (number of chromosomes)	Haplotypes																	
	1 (---)	2 (+---)	3 (--+)	4 (-+--)	5 (-+++)	6 (-++-)	7 (-+-)	8 (-+++)	9 (+++)	10 (---)	11 (--+)	12 (+---)	13 (--+)	14 (+++)	15 (-+-)	16 (-+-)	17* (-+-)	18** (-+-)
Carrier-Sekani, Canada <sup>1</sup> (50)		60.0			18.0	12.0				6.0			4.0					
Mvskoke, USA <sup>1</sup> (70)		45.8			5.7	30.0	1.4	1.4	7.1			1.4	1.4	3.0	1.4	1.4		
Huichol, Mexico <sup>2</sup> (97)		79.2		1.0	2.2				1.0					9.1	1.0			2.0
Yanomámi, Brazil <sup>3</sup> (34)	2.9	91.2				5.9												
Wayampi, Brazil <sup>4</sup> (30)		86.7			3.3	6.7	3.3											
Wayana-Apalai, Brazil <sup>4</sup> (34)		82.4				14.7		2.9										
Wai-Wai, Brazil <sup>5</sup> (56)		87.5				12.5												
Katuena, Brazil <sup>6</sup> (56)		92.8			3.6	3.6												
Arára, Brazil <sup>4</sup> (30)		83.4		3.3		10.0		3.3										
Gavião, Brazil <sup>5</sup> (58)	1.7	87.9	1.7			3.4	5.2											
Zoró, Brazil <sup>5</sup> (60)		93.3				6.7												
Suruí, Brazil <sup>5</sup> (44)		81.8			4.5	11.4				2.3								
Kayapó (Kokraimoro), Brazil <sup>4</sup> (44)		79.5				20.5												
Xavante, Brazil <sup>5</sup> (60)		60.0			15.0	18.3	5.0								1.7			
Asurini, Brazil <sup>6</sup> (14)		78.6			7.1	7.1	7.1											
Awá-Guajá, Brazil <sup>6</sup> (86)		100.0																
Parakanã, Brazil <sup>6</sup> (28)		78.6		7.1		10.7	3.6											
Urubú-Kaapór, Brazil <sup>6</sup> (94)	4.2	70.3		1.1	5.3	11.7		4.2		2.1		1.1						
Zo'é, Brazil <sup>6</sup> (50)		78.0			6.0	16.0												
Kayapó (Xikrin), Brazil <sup>6</sup> (40)		87.5		2.5		10.0												
Tiryó, Brazil <sup>6</sup> (50)		68.0			4.0	18.0		10.0										
Wayuu, Colombia <sup>7</sup> (164)		94.0		1.2	1.2	1.2				1.2		1.2						
Kamsa, Colombia <sup>7</sup> (122)		87.0	1.6			3.3				1.6	1.6	3.3						1.6
Inga, Colombia <sup>7</sup> (28)		93.0										7.0						
Mapuche, Argentina <sup>8</sup> (86)	5.8	57.0	2.3	2.3	3.5	26.7	1.2							1.2				

Haplotype numbering according to Long *et al.* (1990); <sup>1</sup>Mattevi *et al.* (2000); <sup>2</sup>Villalobos-Arámbula *et al.* 2000; <sup>3</sup>Guerreiro *et al.* (1992); <sup>4</sup>Guerreiro *et al.* (1994); <sup>5</sup>Bevilacqua *et al.* (1995); <sup>6</sup>present study; <sup>7</sup>Shimizu *et al.* (2000); <sup>8</sup>Kaufman *et al.* (1998); \*haplotype first described in Melanesians by Hill *et al.* (1988); \*\* haplotype first described in Japanese by Shimizu *et al.* (1992).

type 6 (- + + - +), with frequencies between 7% and 18%. This haplotype was absent only from the Awá-Guajá sample, in which the frequency of haplotype 2 was 100%. Haplotype 5 (- + - + +) was identified in five of the eight tribes, with frequencies between 3.6% and 7%, while haplotype 4 (- + - - +) was observed in three tribes, with frequencies varying from 1% to 7%. The remaining haplotypes were identified in only one or two tribes, although with polymorphic frequencies in some cases.

The haplotype distribution among the tribes showed statistically significant differences ( $\chi^2 = 114.607$ ; D.F. = 56;  $p < 0.001$ ). However, these differences were primarily due to the inclusion of the Awá-Guajá, Urubú-Kaapór and Tiriyo, which presented clear differences with regard to the other tribes. When these three populations were excluded, the haplotype distribution was more homogeneous ( $\chi^2 = 25.032$ ; D.F. = 16;  $p = 0.069$ ).

### Discussion

The most common β-globin gene haplotypes found in Brazilian Amerindians (haplotypes 2, 6, and 5) are considered to be first-order haplotypes in the phylogenetic scheme proposed by Chen *et al.* (1990) and Long *et al.* (1990). These haplotypes were probably brought to America by the first settlers of the continent, given that they are also common in Asia, the probable place of origin of the first migrants, and in the islands of the South Pacific, from where additional immigrants may have come (Salzano and Callegari-Jacques, 1988; Nei and Roychoudhury, 1993; Ward *et al.*, 1991; Horai *et al.*, 1993; Neves *et al.*, 1998). According to this scheme, haplotypes 2, 5, and 6 would have originated directly from the ancestral type, possibly haplotype 1 (Chen *et al.*, 1990 and Long *et al.*, 1990) or haplotype 3 (Vincek *et al.*, 1994), or from another first-order haplotype (1 or 4). First-order haplotypes are separated by at least two mutation or gene conversion events (Long *et al.*, 1990), making it unlikely that they arose in na-

tive Americans from other first-order haplotypes, which are virtually absent in these populations. Second-order haplotypes (7, 9, 11, and 13), derived by recombination from first-order haplotypes, were also found in Brazilian natives at frequencies varying from 1.4% to 10.0. As they are rare or absent in Asians, they probably appeared during the colonization of the American continent. Three other first-order haplotypes (3, 4, and 16) were also found at low frequencies in Brazilian Amerindians. The presence of haplotype 3 can be attributed to admixture with people of African ancestry, since this haplotype is very common in Africans, but rare or absent in all other populations. The presence of the other first-order haplotypes (4 and 16) may also be due to admixture with non-Amerindians, although, as they are also found in Asian and Oceanic populations, they may have been present in the ancestral population.

The distribution of β-globin haplotypes in native Americans is shown in Table 1. To date, eleven different haplotypes have been identified in Brazilian tribes, although most of them have a reduced number of haplotypes (average of 3.5, minimum of 1, and maximum of 8). The highest value was observed in the Urubú-Kaapór, a tribe with significant levels of admixture with non-Amerindians. The haplotype distribution among tribes is generally homogeneous, haplotype 2 accounting for more than 78% of haplotypes. Haplotype 6 accounts for the majority of the remainder, followed by haplotype 5, also found in most of the eighteen tribes studied. Awá-Guajá, Parakanã, Urubú-Kaapór, Tiriyo and Xavante are the exceptions. Haplotype 2 is fixed in the former tribe, while the others present alternative haplotypes with relatively high frequencies.

Genetic variability measured by haplotype diversity and the coefficient of gene differentiation in native American and other ethnic groups is shown in Table 2. Total diversity (Ht) and intrapopulation variation (Hs) observed in Brazilian Amerindians are similar to those estimated for Asians, corroborating the results of Guerreiro *et al.* (1994) and Mattevi *et al.* (2000), but they are higher than those es-

**Table 2** - β-globin gene haplotype diversity in American natives and other human populations.

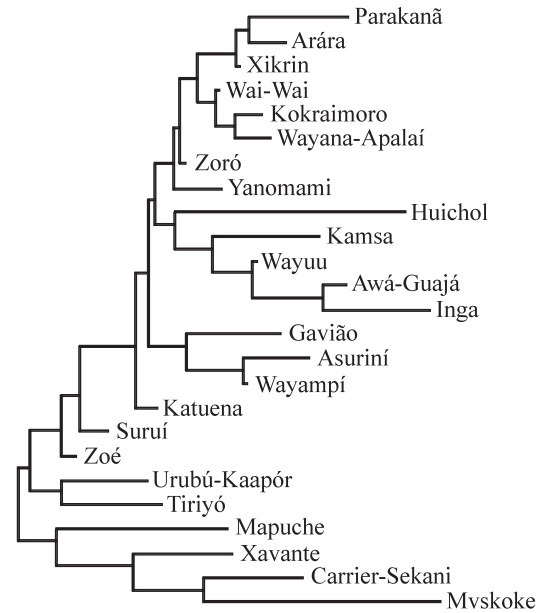
Population	N. of subpopulations	Ht	Hs	Dst	Dm	Gst	Gst'
Africans	6	0.710	0.650	0.060	0.072	0.085	0.100
Europeans	5	0.820	0.590	0.030	0.038	0.280	0.060
Asians	8	0.330	0.310	0.020	0.023	0.061	0.069
North-Americans	2	0.656	0.639	0.018	0.036	0.026	0.053
South-Americans	22	0.306	0.286	0.019	0.020	0.065	0.065
Colombian Amerindians	3	0.164	0.162	0.002	0.003	0.012	0.018
Brazilian Amerindians	18	0.305	0.290	0.015	0.016	0.049	0.052
Brazilian Amerindians <sup>1</sup>	15	0.252	0.244	0.008	0.009	0.032	0.034

Ht, average heterozygosity for the entire population; Hs, average heterozygosity within populations; Dst, interpopulational genetic variation; Dm, average minimum genetic distance among subpopulations; Gst, coefficient of gene differentiation; Gst', coefficient of gene differentiation, considering the number of populations examined.

estimated for Colombian Amerindians and, as expected, lower than those of North-American natives, Africans and Europeans. The interpopulational diversity (D<sub>st</sub>) of Brazilian Amerindians is also higher than that observed in Colombian Amerindians, but a little lower than estimated for native North-Americans and Asians. Some of the Brazilian tribes, such as the Urubú-Kaapór and Tiriyo, and probably the Xavante, present evidence of interethnic admixture and exhibit high levels of heterozygosity, which may account for at least part of these differences. However, Callegari-Jacques and Salzano (1999) estimated interethnic admixture in the Xavante as being only about 2%. Anyway, when these tribes are excluded from diversity analyses, interpopulational diversity in Brazilian Amerindians is reduced to 0.8%, a value lower than those observed in other populations, except for Colombian Amerindians.

The genetic distances (D<sub>A</sub>) between Brazilian Amerindians and other American natives based on haplotype frequencies are shown in Table 3, and their relationship pattern is presented as a dendrogram (Figure 1). As a rule, the genetic distances between Brazilian Amerindians are low, except for the Urubú-Kaapór and Tiriyo, and the genetic affinities among tribes show no clear linguistic or geographic pattern. At a continental level, the lowest values of genetic distances from Brazilian Amerindians were observed in Colombian Amerindians (Wayuu, Kamsa and Inga) and Huichol (Mexico), who cluster in the upper part of the dendrogram (Figure 1). Mvskoke, Carrier-Sekani and Mapuche are the more differentiated groups, and form a separate cluster.

In summary, the results obtained through analysis of the β-globin haplotype in a relatively large number of Brazilian Amerindian populations agree with those of the first studies, indicating a homogeneous distribution of haplo-



**Figure 1** - Dendrogram constructed by the neighbor-joining method, showing the relationships between Amerindian tribes based on β-globin gene cluster haplotype frequencies.

types in most tribes, characterized by a reduced number of haplotypes, and low levels of heterozygosity and differentiation. Haplotype 2 accounts for more than 78% of the total, the rest being mainly accounted for by haplotypes 5 and 6. The Parakanã, Urubú-Kaapór, Tiriyo and Xavante constitute exceptions by presenting at least one other haplotype with a relatively high frequency. The pattern of genetic affinities among Brazilian Amerindians indicates that most groups are closely related to each other, and to Colombian Amerindians, whereas North-American Amerindians are

**Table 3** - Heterozygosity (x100) and genetic distances (D<sub>A</sub>) between Amerindian tribes based on β-globin haplotypes (X 10,000).

	Yan	Asu	Awa	Gav	Pak	Sui	Uru	Wpi	Zoe	Zor	Kok	Xav	Xik	Ara	Kat	Tir	Wai	Wap	Map	Wyu	Ksa	Iga	Car	Mvk	Huc
Yan	16.6																								
Asu	886	38.0																							
Awa	450	1134	0.0																						
Gav	377	589	625	22.4																					
Pak	739	763	1134	652	37.1																				
Sui	543	517	956	898	877	31.8																			
Uru	813	1042	1615	1241	1168	554	48.7																		
Wpi	479	087	689	379	554	319	889	24.5																	
Zoe	594	452	1168	982	862	142	663	296	36.6																
Zor	147	747	341	467	590	390	1016	336	434	12.6															
Kok	385	889	1084	806	614	407	975	526	314	216	32.9														
Xav	1564	365	2254	1439	1309	728	1151	570	499	1411	1157	58.6													
Xik	299	864	646	647	251	472	910	472	474	146	228	1402	22.6												
Ara	511	1061	868	855	385	673	698	678	670	360	426	1573	170	29.7											
Kat	339	448	367	618	839	244	837	194	268	204	552	992	389	603	13.7										
Tir	1094	1026	1754	1486	1301	685	526	860	530	937	727	1023	945	553	872	49.8									
Wai	208	765	646	578	550	346	948	375	324	050	059	1242	132	339	318	786	22.0								
Wap	400	931	923	782	698	496	729	555	449	240	170	1328	296	188	528	349	153	30.0							
Map	1125	1139	2450	1207	1004	1030	819	1094	807	1370	929	972	1064	1196	1392	1208	1111	1166	60.0						
Wyu	475	821	305	708	754	629	1014	490	731	352	859	1597	411	600	245	1321	544	779	1743	11.6					
Ksa	651	1247	673	755	1136	759	1184	845	1036	520	861	1998	701	907	670	1538	633	837	1827	559	24.1				
Iga	790	1450	356	959	1450	1278	1637	1021	1483	685	1401	2530	979	1193	710	2048	979	1246	2719	360	524	13.5			
Car	1761	1079	2254	2099	2000	925	1134	1120	734	1621	1525	875	1659	1831	1076	1294	1530	1640	1568	1158	1782	2001	59.4		
Mvk	2207	1584	3232	2375	1984	1519	1531	1628	1242	2045	1486	1063	1937	1873	1984	1240	1733	1555	1294	2153	2328	3161	949	69.5	
Huc	999	1174	1089	1272	1171	950	1398	916	965	869	1138	1540	866	1040	757	1503	949	1135	1469	761	1138	1407	1537	1607	36.0

Yan, Yanomami; Asu, Asurini; Awa, Awá-Guajá; Gav, Gavião; Pak, Parakanã; Sui, Surui; Uru, Urubú-Kaapór; Wpi, Wayampi; Zoe, Zoé; Zor, Zoró; Kok, Kokraimoro; Xav, Xavante; Xik, Xikrin; Ara, Arára; Kat, Katuena; Tir, Tiriyo; Wai, Wai-Wai; Wap, Wayana-Apalai; Map, Mapuche; Wyu, Wayuu; Kam, Kamsa; Ing, Inga; Car, Carrier-Sekani; Mvk, Mvskoke, Huc, Huichol.

more differentiated and clearly separated from all other tribes, except the Xavante, from Brazil, and the Mapuche, from Argentina.

A restricted pool of ancestral haplotypes may explain the little diversity observed among most present-day Brazilian and Colombian Amerindian groups, while inter-ethnic admixture could be the most important factor accounting for the high number of haplotypes and high levels of diversity observed in some South-American and most North-American tribes.

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