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Brief Communication

D1S80 Locus Variability in Three Brazilian Ethnic Groups

ELISA M. HEIDRICH,[1] MARA H. HUTZ,[1] FRANCISCO M. SALZANO,[1] CARLOS E.A. COIMBRA JR.,[2] AND RICARDO V. SANTOS[2,3]

Abstract We have studied the hypervariable locus D1S80 in 75 white and 53 black Brazilians from Porto Alegre, a southern Brazilian population, and in 50 Brazilian Indians from the Xavante and Zoró tribes. Allele frequencies were significantly different among the three ethnic groups, mainly because of the higher frequencies of alleles **21*, **22*, **28*, and **34* and the lower frequency of allele **18* in individuals of African ancestry and the higher frequencies of alleles **18* and **30* in Brazilian Indians. In the Indian group a restriction in the number of alleles (7) and genotypes (15) was found. The data suggest that the D1S80 locus is a useful interpopulation marker. This is the first report of its allele frequencies in Amerindians.

Since Wyman and White (1980) discovered the first hypervariable locus using an arbitrary DNA probe, over 100 such VNTR (variable number of tandem repeat) loci have been described. The D1S80 locus was identified by Nakamura et al. (1988). Its variation results from the repetition of a 16-bp core sequence (Kasai et al. 1990), and only a few populations have been surveyed in relation to it [United States whites (Budowle et al. 1991); Finland (Sajantila et al. 1992); France (Pfitzinger et al. 1992); Italy (Trabetti et al. 1993); Spain (Gené et al. 1993); United States whites, blacks, and Hispanics (Roche Molecular Systems 1993)].

In this study we present the allele frequencies of the D1S80 locus in two Brazilian Indian tribes and in white and black Brazilians living in Porto Alegre, in southern Brazil. To our knowledge this is the first

[1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brazil.

[2]Departamento de Endemias Samuel Pessoa, Escola Nacional de Saúde Pública, Rua Leopoldo Bulhões 1480, 21041-210 Rio de Janeiro, RJ, Brazil.

[3]Departamento de Antropologia, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, 20940-040 Rio de Janeiro, RJ, Brazil.

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report of allele distributions at this locus in Amerindians. We have also investigated the locus's potential application for paternity determination in these communities.

Materials and Methods

Porto Alegre is the capital of Brazil's southernmost state. The city was founded in 1752 by about 60 white couples from the Azores Islands. At present the whites from Porto Alegre are still mainly of Portuguese descent, but Italians, Spaniards, and Germans have also contributed to the gene pool. Blacks constitute approximately 14% of the population. They are descendants of slaves who were brought to Brazil between the fifteenth and eighteenth centuries, mainly from Africa's west coast but also from Angola and Mozambique.

The white sample consists of 75 individuals who came to the Genetics Department of the Universidade Federal do Rio Grande do Sul for paternity testing. Of the 53 black Brazilians, 50 were ascertained at the Central Laboratory of the General Public Hospital, to which they went for routine blood examinations. The remaining three blacks also came for paternity determinations at the Genetics Department. Twenty-eight of the black Brazilians showed morphological signs of white admixture. Sixteen children from 15 white couples were also typed, to assess the usefulness of AMP-FLP [PCR (polymerase chain reaction) amplified fragment length polymorphism] at this locus.

The Brazilian Indian samples consisted of 50 individuals from the Xavante and Zoró tribes (25 subjects from each group). The populations sampled can be characterized as follows. The Xavante speak a Ge language that can be assigned to the Macro-Ge subdivision of the Ge-Pano-Carib group (Rodrigues 1986; Greenberg 1987). At present, the Xavante live in six different areas delimited by the coordinates 51°10′W–54°5′W, 13°10′S–15°70′S. The village from which material was collected is called Rio das Mortes (51°40′W, 13°20′S) and is situated near the western boundary of the Indian Reservation Pimentel Barbosa, State of Mato Grosso, Brazil. More extensive contacts with non-Indians started in 1946. The Xavante experienced a marked population growth in the last decades; from an estimated number of 1500–2000 in the 1960s, the Xavante population grew to the present 6233 individuals (Ricardo 1991; Flowers 1994).

The Zoró language is classified in the Tupi stock, Mondé family (Rodrigues 1986). The Zoró live in a single village located 20 km from the Rio Branco (approximately 60°20′W, 10°20′S), Aripuanã Park, State of Mato Grosso, Brazil. More extensive contacts with non-Indians started only in 1977, and at that time it was estimated that the population num-

bered 350 persons. By August 1990, however, this number had been reduced to 215 (Santos 1991).

High-molecular-weight DNA was isolated from 3 ml of whole blood by the salting-out procedure of Miller et al. (1988). PCR analysis was performed using the D1S80 Forensic DNA Amplification Reagent kit (Perkin-Elmer) under conditions recommended by the manufacturer. Allele identification was achieved by comparison with the allelic ladder provided with the reagent set.

Hardy-Weinberg equilibrium was tested using a binomial test (Zar 1984, pp. 287–290; nominal level 0.05, two-sided), as proposed by Odelberg et al. (1989) for VNTR when the relative expected frequencies are small. Interpopulation comparisons were made with the chi-square test. The probability of exclusion of a given person in paternity evaluations using this system was calculated according to methods described by Roychoudhury and Nei (1988). The probability that two randomly selected individuals have the same genotype (probability of matching) was calculated as described by Risch and Devlin (1992).

Results

The genotypes identified in black and white Brazilians are listed in Table 1, and those of Brazilian Indians are given in Table 2. Allele designation was based on the number of repeats of the core sequence. The total number of genotypes observed were 32 and 34 for white and black Brazilians, respectively; in Brazilian Indians only 15 genotypes were identified (8 in the Zoró and 13 in the Xavante).

The most common genotypes in white Brazilians were **18/*18*, **18/*24*, and **24/*24*. These three genotypes represent 48% of the individuals typed. In the black Brazilian sample genotype **24/*24* was also the most frequent, but several other genotypes were found in relatively high proportions (see Table 1). In Brazilian Indians genotypes **18/*30*, **18/*18*, and **30/*30* accounted for 56% of the individuals screened, but their prevalences were different in the two tribes studied (see Table 2). The observed heterozygosity is higher in black Brazilians (74%) than in white Brazilians or Brazilian Indians (67% and 66%, respectively).

The genotype frequencies observed in black Brazilians and in Brazilian Indians are in agreement with those expected under Hardy-Weinberg equilibrium. In the white sample, however, there is a slight deviation ($p < 0.05$), namely, a small excess of the **18/*18* and **24/*24* homozygotes.

Allele distributions are shown in Table 3. Black Brazilians are characterized by higher frequencies of alleles **21*, **22*, **28*, and **34* but a lower frequency of allele **18* compared with white Brazilians. In Bra-

Table 1. Distribution of Genotypes at the D1S80 Locus in Unrelated Whites and Blacks from Porto Alegre, Brazil

	Whites		*Blacks*	
Genotype	*N*	*%*	*N*	*%*
**16/*28*	1	1.3		
**17/*18*			1	1.9
**17/*25*	1	1.3	1	1.9
**17/*29*			1	1.9
**18/*18*	10	13.3		
**18/*21*	1	1.3	1	1.9
**18/*22*	2	2.7	1	1.9
**18/*23*	2	2.7		
**18/*24*	11	14.7	1	1.9
**18/*26*	1	1.3		
**18/*27*	1	1.3		
**18/*28*	1	1.3	2	3.8
**18/*29*	2	2.7		
**18/*32*	1	1.3		
**18/*34*			1	1.9
**19/*20*			1	1.9
**21/*21*			1	1.9
**21/*23*	1	1.3		
**21/*24*	1	1.3	2	3.8
**21/*25*	1	1.3		
**21/*27*			1	1.9
**21/*28*			1	1.9
**21/*29*	1	1.3		
**22/*22*			2	3.8
**22/*24*			2	3.8
**22/*28*	1	1.3	2	3.8
**22/*30*			1	1.9
**22/*34*			1	1.9
**23/*23*			1	1.9
**23/*29*	2	2.7		
**23/*34*			1	1.9
**23/*38*	1	1.3		
**24/*24*	15	20.0	6	11.3
**24/*25*	3	4.0	3	5.6
**24/*28*	1	1.3	3	5.6
**24/*29*	3	4.0	1	1.9
**24/*30*	1	1.3		
**24/*31*	1	1.3	3	5.6
**24/*32*			1	1.9
**24/*33*			1	1.9
**24/*34*	1	1.3	2	3.8
**24/>*41*	1	1.3		
**25/*28*	1	1.3		
**25/*30*	1	1.3		
**25/*31*	2	2.7		
**26/*31*	2	2.7		
**26/*34*			1	1.9

Table 1. Continued

	Whites		*Blacks*	
Genotype	*N*	*%*	*N*	*%*
**28/*28*			1	1.9
**28/*29*	1	1.3		
**28/*31*			1	1.9
**28/*34*			1	1.9
**29/*33*			1	1.9
**34/*34*			3	5.6
Total number of genotypes	32		34	

zilian Indians the most prevalent alleles are **18* and **30*. Allele **24*, the most frequent allele in white (0.35) and black Brazilians (0.29), shows a frequency of only 0.10 in the Brazilian Indians.

To investigate the usefulness of the D1S80 locus for paternity determinations in Brazil, we estimated the probability of exclusion of a falsely accused individual using only the D1S80 system. The probabilities were 60%, 73%, 53%, and 32% in white Brazilians, black Brazil-

Table 2. Distribution of Genotypes at the D1S80 Locus in Two Brazilian Indian Tribes

	Zoró		*Xavante*		*Total*	
Genotype	*N*	*%*	*N*	*%*	*N*	*%*
**18/*18*	1	4.0	6	24.0	7	14.0
**18/*24*	1	4.0	2	8.0	3	6.0
**18/*25*	1	4.0	4	16.0	5	10.0
**18/*26*			1	4.0	1	2.0
**18/*28*	1	4.0			1	2.0
**18/*30*	11	44.0	3	12.0	14	28.0
**24/*24*			1	4.0	1	2.0
**24/*25*			1	4.0	1	2.0
**24/*30*	2	8.0	1	4.0	3	6.0
**24/*31*			1	4.0	1	2.0
**25/*26*			1	4.0	1	2.0
**25/*30*	1	4.0	1	4.0	2	4.0
**25/*31*			1	4.0	1	2.0
**30/*30*	7	28.0			7	14.0
**31/*31*			2	8.0	2	4.0
Total number of genotypes	8		13		15	

Table 3. Allele Frequencies at the D1S80 Locus in the Three Ethnic Groups Investigated

Allele	White Brazilians (N = 150)	Black Brazilians (N = 106)	Brazilian Indians		
			Zoró (N = 50)	Xavante (N = 50)	Total (N = 100)
*16	0.007	0.000	0.000	0.000	0.000
*17	0.007	0.028	0.000	0.000	0.000
*18	0.280	0.066	0.320	0.440	0.380
*19	0.000	0.009	0.000	0.000	0.000
*20	0.000	0.009	0.000	0.000	0.000
*21	0.033	0.066	0.000	0.000	0.000
*22	0.020	0.104	0.000	0.000	0.000
*23	0.040	0.028	0.000	0.000	0.000
*24	0.353	0.293	0.060	0.140	0.100
*25	0.060	0.038	0.040	0.160	0.100
*26	0.020	0.009	0.000	0.040	0.020
*27	0.007	0.009	0.000	0.000	0.000
*28	0.040	0.113	0.020	0.000	0.010
*29	0.060	0.028	0.000	0.000	0.000
*30	0.013	0.009	0.560	0.100	0.330
*31	0.033	0.038	0.000	0.120	0.060
*32	0.007	0.009	0.000	0.000	0.000
*33	0.000	0.019	0.000	0.000	0.000
*34	0.007	0.123	0.000	0.000	0.000
*38	0.007	0.000	0.000	0.000	0.000
>*41	0.007	0.000	0.000	0.000	0.000

N = Number of chromosomes examined.

Chi-square values for the differences: white vs. black Brazilians, 43.6 (5 d.f., $p < 0.001$); white Brazilians vs. Brazilian Indians, 77.4 (5 d.f., $p < 0.001$); black Brazilians vs. Brazilian Indians, 109.4 (7 d.f., $p < 0.001$). Classes with a small number of observations were pooled in these comparisons.

ians, Xavante Indians, and Zoró Indians, respectively. The probability that two randomly selected individuals have the same genotype (probability of matching) was estimated as 0.072, 0.031, 0.12, and 0.29, respectively.

In 15 cases of disputed paternity among white Brazilians, we blindly compared the results of the D1S80 locus with those of 15 protein polymorphisms. In one case there were two children, and in another case there were two putative fathers. In seven cases the D1S80 paternal allele was absent in the child and the putative fathers were therefore excluded. Six of the cases were also excluded with the protein markers, the remaining giving, with them, a low probability of paternity (56%).

Discussion

Most published D1S80 data are from white populations. In this ethnic group the most prevalent alleles are **24* and **18*, their sum ranging from 55% in France to 63% in Brazil. For the allele frequencies in general, however, the differences among the white populations are small and statistically nonsignificant, the exception being the Finnish sample, which shows departures in the prevalences of several alleles. No significant differences were observed between the black Brazilians studied here and black Americans (Roche Molecular Systems 1993) either.

The marked reduction in variability observed in Brazilian Indians regarding the D1S80 locus is worth noting. This is especially true for the Zoró, where the probability of matching is 9.4 times higher than that for black Brazilians. Previous reports about decreases of diversity among Amerindians have involved mostly the HLA system at the serological level [see, for instance, Kostyu and Amos (1981)]. The reduction is less marked for non-VNTR RFLPs (restriction fragment length polymorphisms) (Kidd et al. 1991). Kidd et al. also observed decreases in the heterozygosities of seven VNTR systems that did not include the D1S80 locus. They indicated some problems in heterozygosity estimations involving VNTR systems, but their remarks do not apply to the D1S80 locus. Further data are needed before we can start discussing the *causes* of these heterozygosity differences.

The interpopulation variability registered here emphasizes the need for proper investigation of local peculiarities in the forensic application of the D1S80 system. For instance, Pena and Chakraborty (1994), without presenting allele frequencies in detail, reported a heterozygosity of 84% for a sample of 448 southeastern white Brazilians, a value that is different from the 67% obtained here. On the other hand, the frequency of 7% observed for allele **30* among 210 US Hispanics (much higher than the frequencies obtained in other white populations) may reflect Amerindian admixture in this sample.

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