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ISOELECTRIC FOCUSING STUDIES IN BRAZILIAN INDIANS —UNCOVERING VARIATION OF ORM, AHSG AND IF—

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Summary Allele frequencies for the orosomucoid 1 (ORM1), orosomucoid 2 (ORM2), alpha-2-HS-glycoprotein (AHSG) and complement component I (IF) loci were studied in 393 individuals of three Brazilian Indian tribes. In the ORM1 locus only two alleles were observed among the Urubu-Kaapor, while five were found among the Pacaás Novos. The frequency of *ORM1*1* was similar in these two tribes (0.734 and 0.715, respectively) but departed more markedly among the Parakanã (0.870). Variation for ORM2 locus was found among the Pacaás Novos only, with *ORM2*3* being observed in just three individuals. A new variant (*AHSG*PN*) was found in the AHSG system. Frequency for *AHSG*1* was unexpectedly low in the three tribes, especially, among the Pacaás Novos, where the prevalence (0.145) is the lowest considering other data reported thus far. For IF locus, variability was also restricted to only one trible (Urubu-Kaapor) and attributed to a new polymorphic allele, *IF*A3*.

Key Words polymorphism, Brazilian Indians, ORM1 and ORM2, AHSG, IF

INTRODUCTION

Technological improvements are making possible the discovery of enormous amounts of variability in the gene pool of human and other species. The necessary knowledge about the variations in different populations of the world, however, is lagging behind. This is due to several causes, two of the most important being

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the present emphasis on linkage studies involving mainly pathological traits, and the difficulty of establishing in a given center all the techniques necessary for these investigations. It is important to change this trend because to really understand the mechanisms involved in the causes of these variation markers influencing processes at different levels of the biological spectrum (DNA, proteins of different types) should be studied. The implication is that the information available for traditional systems should be complemented with that involving new ones, as additional research tools develop.

The present report presents results on the orosomucoid 1 (ORM1), orosomucoid 2 (ORM2), alpha-2-HS-glycoprotein (AHSG) and complement component I (factor I, IF) loci for three Brazilian Indian tribes, already studied for around 30 other systems (Hamel *et al.*, 1984; Salzano *et al.*, 1985; Black *et al.*, 1988). The information available for ORM1 and ORM2 is thus extended, and the first results for AHSG and IF in unmixed Amerindians are given, with the concomitant discovery of new variants. After integrating these old and new data some features of the world distribution of these markers will be discussed.

MATERIALS AND METHODS

The groups studied can be characterized as follows (see Fig. 1 for their geographical location):

(1) Urubu-Kaapor: they speak a Tupi language and are settled on a reservation located between 46° to 47°W and 2° to 3°S in the Brazilian State of Maranhão. Total population is 710 persons. Peaceful contacts with non-Indians were started in 1928, but despite this relatively long interaction they still keep much of their culture (Samain, 1984/85; Ricardo, 1986). The samples were collected in seven villages scattered along the reservation, but since the number obtained in each was small they were pooled for this analysis. Previous genetic studies among them were reported by Black *et al.* (1988).

(2) Parakanã: they also speak a Tupi language. The present sample was collected at the village Bom Jardim (5°55′S, 52°42′W), which had a total population, in 1984, of 133. Other members of this tribe live in two other villages with 139 and 72 inhabitants, respectively. First contacts with non-Indians started in the 1970s only. Other genetic and cultural information about them can be found in Black *et al.* (1980, 1988).

(3) Pacaás Novos: their language can be classified in the Chapacura stock. The approximately 1,000 persons live in several communities in the Brazilian State of Rondonia, near the Bolivian border. The samples were obtained at the villages of Tanajura (11°5′S, 65°10′W), at the margins of the Pacaás river, and Santo André, located about 40 km up river. Contacts of a more permanent nature with non-Indians started in 1961. Further details about the demography, culture and genetics of these Indians can be obtained in Hamel *et al.* (1984) and Salzano *et al.*

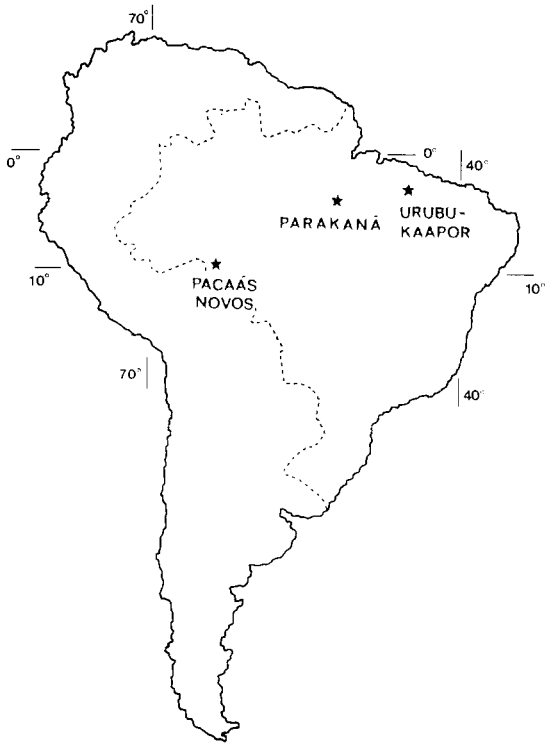

Fig. 1. South America's map showing where the studied populations live.

(1985).

The ORM typing was carried out by polyacrylamide gel isoelectric focusing (IEF) followed by immunoprinting (Yuasa *et al.*, 1986; Umetsu *et al.*, 1989a). The AHSG typing was determined by polyacrylamide gel IEF followed by immunoblotting (Yuasa and Umetsu, 1988). The IF typing was carried out by agarose IEF followed by immunoblotting (Ding *et al.*, in press).

RESULTS

Table 1 presents the phenotype prevalences, and Table 2 the allele frequencies for the four loci studies. There is little intratribal differentiation among the Pacaás Novos, and the results concerning them will be examined considering the tribe as a whole.

In the ORM1 locus only two alleles were observed among the Urubu-Kaapor, but as many as five including two duplicated alleles (*ORM1*2•1* and *ORM1*5•2*) among the Pacaás Novos. The frequency of *ORM1*1* among these two tribes was similar to each other (0.734 and 0.715, respectively), but differed markedly from

Table 1. Phenotype prevalences considering four loci investigated in three Brazilian Indian tribes.

Phenotypes		Urubu-Kaapor	Parakanã	Pacaás Novos		
				Tanajura	Santo André	Total
ORM1	1	41	88	44[a]	55	99
	2-1[b]	31	17	27	38	69
	2	5	1	2	6	8
	2•1-1	0	9	1	2	3
	2•1-2	0	1	0	0	0
	5-1	0	0	2	0	2
	5-2[c]	0	0	0	2	2
	5•2-1	0	0	10	8	18
	5•2-2	0	0	2	1	3
ORM2	1	77	116	85	112	197
	3-1	0	0	3[a]	0	3
AHSG	1	11	35	3	6	9
	2-1	37	55	19	21	40
	2	29	26	63	85	148
	PN-2	0	0	3	0	3
IF	B	59	116	88	112	200
	A3-B	18	0	0	0	0

[a] The three samples had the *ORM1*1-ORM2*3* haplotype. [b] Including two ORM1 (2/1 and 2•1/2•1) genotypes. [c] Including two ORM1 (5/2 and 5•2/5•2) genotypes.

Table 2. Allele frequencies in four loci of three Brazilian Indian tribes.

Alleles	Urubu-Kaapor	Parakanã	Pacaás Novos		
			Tanajura	Santo André	Total
*ORM1*1*	0.734	0.870	0.727	0.705	0.715
*ORM1*2*	0.266	0.085	0.188	0.236	0.213
*ORM1*2•1*	—	0.045	0.006	0.009	0.007
*ORM1*5*	—	—	0.011	0.008	0.009
*ORM1*5•2*	—	—	0.068	0.042	0.056
*ORM2*1*	1.000	1.000	0.983	1.000	0.992
*ORM2*3*	—	—	0.017	—	0.008
*AHSG*1*	0.383	0.539	0.142	0.147	0.145
*AHSG*2*	0.617	0.461	0.841	0.853	0.847
*AHSG*PN*	—	—	0.017	—	0.008
*IF*B*	0.883	1.000	1.000	1.000	1.000
*IF*A3*	0.117	—	—	—	—

the data in the Parakanã (0.870). Variation for ORM2 was found among the Pacaás Novos only, with *ORM2*3* being observed in just three among 200 individuals.

As for the common alleles in the AHSG system, the Pacaás Novos show an extremely low frequency of *AHSG*1* (0.145), the prevalences among the Urubu-Kaapor and Parakanã being 0.383 and 0.539, respectively, also quite low. As shown in Fig. 2, a new AHSG variant was observed in the Pacaás Novos population. The variant in the native state was indistinguishable from AHSG 16 and in the desialylated state was indistinguishable from AHSG 2. The AHSG 16 was located between AHSG 1 and AHSG 2 (Fukuma *et al.*, 1990). A family data was available to show a codominant inheritance (Fig. 4).

For IF, variability again was restricted to just one tribe (Urubu-Kaapor),

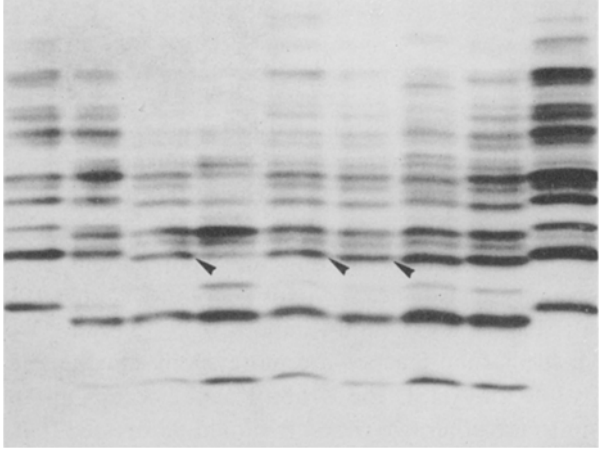

Fig. 2. Immunoblotted patterns of AHSG after IEF of native samples. Anode is at the top. Phenotypes from left to right: AHSG 1, 2-1, PN-2 (mother), 2 (father), PN-2 (twin), PN-2 (twin), 8-2 (control), 16-2 (control) and 1. Filled triangles indicate major variant bands.

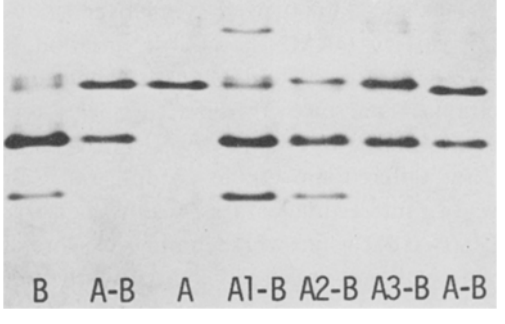

Fig. 3. Immunoblotted patterns of IF phenotypes. Anode is at the top.

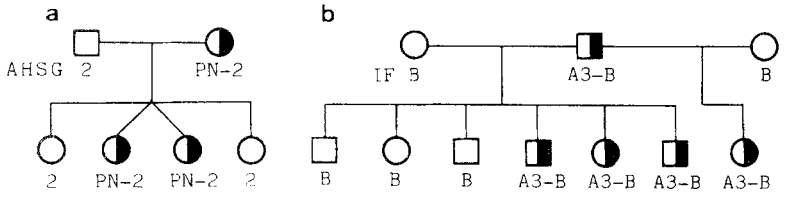

Fig. 4. Pedigrees of families with AHSG PN (a) and IF A3 (b). The twins who are carriers of the AHSG PN variant are identical in 32 genetic systems (probability of dizygosis, less than one in 1,000).

but in this case the new allele found (*IF*A3*) occurs in polymorphic frequencies. The new variant band migrated between IF A and IF A2 (Fig. 3). Pedigrees demonstrating the codominant inheritance of IF A3 are shown in Fig. 4.

DISCUSSION

How are the present results compared with other information available for these three tribes? The Urubu-Kaapor and Parakanã speak languages of the same stock and are closer geographically to each other than to the Pacaás Novos, who speak a very distinct language. These relationships, however, were not reflected in the genetic distances separating these populations, as calculated using 60 alleles of 20 genetic systems; Pacaás Novos—Urubu-Kaapor, 0.20; Pacaás Novos —Parakanã, 0.49; Parakanã—Urubu-Kaapor, 0.55 (Salzano *et al.*, 1990). In the ORM1 system the Urubu-Kaapor are more similar to the Pacaás Novos than to the Parakanã, while for AHSG the Urubu-Kaapor occupy an almost equidistant position in relation to the other two tribes. It should be stressed that the Prakanã are unusually inbred, while the Urubu-Kaapor may have incorporated persons from other tribes.

The amount of information available on the world distribution of the alleles at the four loci varies markedly. Most of the data have been reviewed by Roychoudhury and Nei (1988), Yuasa and Umetsu (1988) and Umetsu *et al.* (1988, 1989a, b). Around 6,000 and 11,000 persons all over the world were tested for AHSG and ORM1, respectively. ORM2 shows little variation, but *ORM2*6* presents prevalences around 4% in Japanese and Chinese (Umetsu *et al.*, 1988). When variability occurs, the range is narrow with different alleles represented in different populations. Only few studies were done for IF (Yuasa *et al.*, 1988). Variation at AHSG does not differentiate ethnic groups well. But the variability at the ORM1 locus shows two interesting features: (a) the high frequencies of *ORM1*1* in Asiatics (values of 0.68–0.81) while whites and blacks present lower prevalences (many lower than 0.65) with not much differentiation between them; and (b) a clear north-south distinction in Asia, with lower frequencies of *ORM1*1* and higher of *ORM1*2-1* in the north. *ORM1*2-1*, also, is present at low frequencies in

two of the three Brazilian Indians.

The most marked features of the present results are: (a) the striking low frequencies of *AHSG*1* (0.145–0.539), while previous results indicated a worldwide range of values between 0.56 and 0.84; and (b) the new polymorphism observed at the IF locus. Since *IF*A3* is at present restricted to the Urubu-Kaapor, it can be provisionally labeled as a "private" polymorphism (Tanis *et al.*, 1974, 1977). *IF*A*, which occurs with polymorphic frequencies in the various Mongoloid populations (Nakamura and Abe, 1985; Yuasa *et al.*, 1988; Ding *et al.*, in press), was not encountered in our present material.

Only the ORM loci have been previously studied among South American Indians. Escallon *et al.* (1987) reported no variation at the ORM2 locus and a frequency of 0.56 of *ORM1*1* among 62 Huitoto Indians of Colombia. This frequency is significantly lower than those observed in the three tribes, where the range in 0.715–0.870 with an average of 0.77 (χ^2: 25.5; 1 d.f.; $p<0.001$). The observations in North American and Mexican Indians also indicate low prevalences of *ORM1*1* (Dogrib, n=169: 0.55, Escallon *et al.*, 1987; Mexican n=24, 0.54, Johnson *et al.*, 1969; Mayan, n=62, 0.62; Escallon, 1987). The comparison of the findings between North and South American Indians, however, is difficult because of differences in methodology; for instance, among North American Indians *ORM1*2•1*, *ORM1*5* and *ORM1*5•2* were not differentiated.

As for the AHSG system, two admixed populations with large amounts of Amerindian parentage have been studied. In both the frequency of *AHSG*1* is that expected considering our values (0.145–0.539) and the fact that non-Indian groups generally have higher prevalences of the gene (Mexican-Americans of Starr County, Texas, n=733, 0.64, Hewett-Emmett *et al.*, 1986; Paraguayans from Asunción, n=200, 0.56, Umetsu *et al.*, 1989b).

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