Apolipoprotein B Signal Peptide Polymorphism Distribution among South Amerindian Populations

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Abstract We report the distribution of the *APOB* signal peptide polymorphism in 5 native populations of South America: 2 samples of Mataco and 1 sample each of Pilagá and Toba from the Argentinian Chaco and 1 sample of Ache from the Paraguay forest. A randomly selected subsample of a previously studied sample from the Cayapa of Ecuador (Scacchi et al. 1997) was reanalyzed to investigate probable differences attributable to sampling, laboratory techniques, or interobserver error. The polymorphism observed in the signal peptide region of the *APOB* gene among native populations of South America exhibits the same range of variation found among geographic continental populations, confirming the high genetic heterogeneity of South Amerindians. Extremes in the allele prevalences were found among the Mataco and Ache, populations not far apart geographically. The small differences in genotype and allele frequencies between the subsample of the Cayapa analyzed here and the original Cayapa sample and between the 2 Mataco samples were not statistically significant and most likely were due to sampling error.

Apolipoprotein B is a monomeric glycoprotein that plays a central role in lipoprotein metabolism and in maintaining the normal homeostasis of serum

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cholesterol levels (Sparks and Sparks 1985; Peacock et al. 1992). The gene coding for human apolipoprotein B (*APOB*) is 43 kb in length with 81 bp coding for a 27–amino acid signal peptide. This terminal signal sequence directs the emerging protein to translocate through the endoplasmic reticulum membrane. The signal peptide of human *APOB* is of variable length. This polymorphism was first typed directly using the polymerase chain reaction (PCR) by Boerwinkle and Chan (1989). The common alleles are the insertion allele (93 bp), which contains a 27–amino acid signal peptide, and the deletion allele (84 bp), with a 24–amino acid signal peptide resulting from the deletion of leucine-alanine-leucine (Boerwinkle et al. 1991). A significant association was detected between the insertion-deletion polymorphism and plasma glucose levels in Europeans and Mexican Americans (Boerwinkle et al. 1991). Some studies have also demonstrated correlations between the insertion-deletion polymorphism and lipid levels (Hansen et al. 1993; Saha et al. 1993; Visvikis et al. 1993; Wu et al. 1994).

Anderson et al. (1997) indicated that *APOB* polymorphism affects interindividual variation in serum lipoprotein and lipid levels in African populations. Besides the insertion and deletion alleles, a 99-bp rare allele was detected in African blacks (Anderson et al. 1997), Caucasians (Hixson et al. 1992), and Mexican Americans (Boerwinkle et al. 1990). This allele contains a 29–amino acid signal peptide as a result of the addition of 2 leucines in a region that normally has 6 identical codons for leucine (Hixson et al. 1992). In addition, the existence of 2 other alleles (87 bp and 102 bp) was reported in a large sample of Japanese [S. Naganawa, communication to Genome Data Base (www.gdb.org), 1992].

Although the *APOB* signal peptide polymorphism has been extensively studied in different ethnic groups, there is little information about its distribution among Amerindians. In this study we report the distribution of the *APOB* signal peptide polymorphism in 5 native populations of South America: the Mataco (2 samples), the Pilagá, and the Toba from the Argentinian Chaco and the Ache from the Paraguayan forest. Included in the analysis is a subsample of a previously studied sample from the Cayapa of Ecuador (Scacchi et al. 1997). Approximately half of the individuals from the original sample were chosen randomly to investigate probable differences attributable to sampling, laboratory technique, or interobserver error.

Materials and Methods

The Mataco (or Wichí), the Toba, and the Pilagá inhabit the southeastern part of the Gran Chaco, a subtropical region that occupies a portion of Paraguay, Bolivia, and northeastern Argentina. Their traditional way of life is based on fishing, hunting, and the gathering of different wild products, although today many migrate for employment to the sugar plantations and other

occasional jobs. The Mataco belong to the Mataco linguistic stock, whereas the Toba and the Pilagá are Guaycuru-speaking tribes (Loukotka 1968). The blood specimens studied for 1 of the Mataco samples and the other 2 tribes were sampled in different settlements located in the province of Formosa, Argentina. An additional Mataco population was also ascertained in Santa Vitoria Este, Department of Rivadavia, Salta Province, Argentina.

The Ache (also known as the Guayaki, a term that the Ache consider offensive) are 1 of the most southerly representatives of the tropical forest Tupi linguistic stock. They reside in the Alto Paraná area of eastern Paraguay in 5 mission/reservation settlements (Hill and Hurtado 1996). The samples for the present study were obtained at the Arroyo Bandera and Chupa Pou reservations. As clearly characterized by Brown et al. (1974), the Ache present an external phenotype uncommon for Amerindians. They are surprisingly fair and of short stature, and many males are prematurely bald, a rare trait for American Indians. They have been extensively studied from the demographic, medical, and ecological points of view, and a recent evaluation of their life history was conducted by Hill and Hurtado (1996).

The Cayapa live along the Cayapas River and its tributaries in northwestern Ecuador (Scacchi et al. 1997). As classified by Loukotka (1968), they speak a Chibchan language that belongs to the northern Andean linguistic division. The Cayapa lifestyle and dietary habits remain almost unaltered by Western contacts. Their diet consists of fruit, vegetables, and products of hunting and fishing (Scacchi et al. 1997).

Genomic DNA was extracted from whole blood using both the salting-out procedure of Miller et al. (1988) and the QIAamp blood kit (Qiagen Inc.). Genotypes were determined by means of PCR using the primers and protocol described by Anderson et al. (1997). For each sample approximately 50–100 ng of DNA was amplified in a total volume of 25 μl. The polymorphisms were directly visualized on 3% agarose gels [1/3 NuSieve 3:1 and 2/3 seaKEM (FMC)] after being stained with ethidium bromide.

A chi-square goodness-of-fit test was used to test the fit of the observed genotype frequencies to those expected under Hardy-Weinberg equilibrium. Population differences in genotype frequencies were tested for significance by contigency chi-square tests. We follow the nomenclature adopted by Boerwinkle et al. (1991) because it is independent of the typing technique used (such as the position of the amplifying oligonucleotides) and because it makes no assumption about the mechanisms of origin of the alleles, therefore easily allowing for the naming of additional variants.

Results and Discussion

The distribution of the *APOB* signal peptide genotypes and allele frequencies in the populations studied are presented in Table 1. The rare

Table 1. Distribution of the *APOB* Signal Peptide Polymorphism in Several South Amerindian Populations

5′βSP Genotype and Allele	*Population*						
	Mataco 1[a]	*Mataco 2*[b]	*Toba*	*Pilagá*	*Ache*	*Cayapa 1*[c]	*Cayapa 2*[d]
Genotype							
**24/*24*	0	0	0	6	7	10	18
**24/*27*	13	14	10	18	38	18	47
**27/*27*	26	29	17	19	12	20	31
**27/*29*	2	0	0	2	0	0	0
Alleles (%)							
**24*	16	16	19	33	46	40	43
**27*	82	84	81	65	54	60	57
**29*	2	0	0	2	0	0	0

a. Salta Province.
b. Formosa Province.
c. Subsample analyzed in this study.
d. Total sample described by Scacchi et al. (1997).

*5′βSP*29* allele was found in only 2 Pilagá and 2 Mataco individuals, both presenting the same genotype (**27/*29*). All the other specimens show the 2 more frequent variants previously reported for other populations. The genotype distributions do not show significant departure from Hardy-Weinberg equilibrium, except among the Ache ($p = 0.009$) because of an excess of heterozygotes. This may be a sampling effect.

The allele distribution of the Mataco and the Toba is similar to distributions found among the Chinese (Saha et al. 1992, 1993) and Japanese [S. Naganawa, communication to Genome Data Base (www.gdb.org), 1992], which is expected when the Asian origin of Amerindians is considered. On the other hand, the allele frequencies observed in the Pilagá, Cayapa, and Ache fall within the range observed in Europeans (Xu et al. 1990; Boerwinkle et al. 1991; Hansen et al. 1993) and Mexican Americans (Boerwinkle et al. 1990). The intergroup chi-square comparisons showed significant differences at the 1% level between the Ache and the 3 populations of the Gran Chaco and between the Cayapa and the Mataco, whereas between the Pilagá and the Mataco, the Cayapa and the Toba, and the Cayapa and the Ache the differences are at the 5% level. This wide range of variation, even among the geographically and linguistically related populations of the Gran Chaco, suggests either non-Indian admixture in some of the populations or, considering the small sample sizes, a sampling artifact. However, if the extremely high genetic variation observed previously in South American aboriginals is taken into account, this result does not appear problematic (Kidd et al. 1991; Cavalli-Sforza et al. 1994).

Additional comparisons can be made with the results of Kaufman et al. (1999), in which *APOB* polymorphism is reported for 5 Brazilian Indian tribes. The studied groups can be clearly separated into northern (Cayapa, Wai Wai, Surui, Zoró, Gavião, Xavante) and southern (Mataco, Ache, Toba, Pilagá) sets. However, no significant differences in average allele frequencies are observed when these 2 sets are compared (*5′βSP*27*: 69% and 73%, respectively), nor is there any clear geographic gradient. As a matter of fact, the extreme values in the allele prevalences occur between groups that are geographically not far apart: the Ache (*5′βSP*27*: 57%) and the Mataco (*5′βSP*27*: 82–84%). The rare *5′βSP*29* allele occurs in both the north and the south.

The small differences in genotype and allele frequencies observed between the subsample of the Cayapa analyzed here and in the original sample described by Scacchi et al. (1997) and between the 2 Mataco samples are not significant and most likely are due to sampling error. Because the amplification of this DNA fragment (less than 100 bp) and its characterization on agarose gels is a relatively simple procedure, the possibility of errors related to laboratory techniques or human handling should be negligible.

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