Brief Communication: Molecular Characterization of O Alleles at the ABO Locus in Chilean Aymara and Huilliche Indians

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ABSTRACT A molecular characterization of alleles O^1, $O^{1variant}$ (O^{1v}), and the mutation G542A of the ABO blood group was performed in two Amerindian populations of Chile, the Aymara ($n = 84$) and the Huilliche ($n = 75$). In addition, a sample of 82 individuals of Santiago belonging to the mixed Chilean population was typed for comparative purposes. The polymorphisms which allow for molecular differentiation of different alleles of the O blood group were studied in genomic DNA. The mutations G188, G261−, G542A, T646A, and C771T, described for alleles O^1, O^{Iv}, and G542A, were determined using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. All individuals studied were group O homozygotes for the deletion G261−, which defines the O^1 alleles. Results obtained indicate that allele O^{1v} exhibits frequencies of 0.65, 0.81, and 0.60 in Aymara, Huilliche, and Santiago populations, respectively. The frequencies of allele $O^{1(G542A)}$ were 0.119, 0.113, and 0.079 in the same populations. Frequencies for alleles O^1 and O^{1v} obtained in the Chilean populations studied concur with the results obtained by other authors, respecting the greater frequency of allele O^{1v} as well as with its heterogeneous distribution in aboriginal South American populations. In Chilean populations, Allele G542A exhibits lower frequencies than those described for indigenous populations from Brazil and may be used as an Amerind admixture marker.

With the advent of new techniques and procedures in the field of molecular biology, new sources of research with interesting projections have opened up to bioanthropology. Among them, the molecular characterization of allelomorphic variants of blood group systems stands out, among which the ABO blood group deserves special mention due to its peculiar geographic distribution. As is already known, this system was discovered by Karl Landsteiner in the year 1900. It is the most important blood group system in transfusional medicine. The genetic and serologic characteristics of this system, as well as the biosynthesis of the ABO antigens, have been well established (Watkins, 1966; Yamamoto et al., 1990; Bennett et al., 1995).

The genetic basis which sustains the existence of different antigenic specificities (antigens A, B, and O) corresponds to mutations in the sequence of the gene which codifies for the glycosyltransferase involved in the addition of an H substance specific sugar codified by another gene along the route. These changes are generated mainly in exons 6 and 7 of the ABO gene sequence, where 77% of the protein is codified and 91% of the catalytic domain of this enzyme is found.

The ABO*O (O^1) allele differs from alleles ABO*A and ABO*B in a mutation of exon 6 of the sequence of the ABO gene which corresponds to a deletion of a G (guanine) nucleotide, in the 261 position (G261−). This mutation, which generates a change within the reading frame of the protein, and is therefore a nonfunctional product, is a characteristic of the great majority of O alleles described so far. There exists a small proportion of O alleles that cannot be defined on the basis of a mutation present in nucleotide 261. From these variants, allele O^2 stands out, presenting some mutations which would generate an aminoacidic change in a key domain of the protein, consequently producing the already mentioned allele. The description and characterization of the polymorphisms would be useful, however, for assigning a determined nomenclature to this variant (Franco et al., 1995; Yip, 2002).

Different O alleles have been described in several populations; the two most frequent of these correspond to alleles O^1 and $O^{1variant}$ (O^{1v}); both have the deletion G261−. Allele O^1 corresponds to the classical allele, whereas allele O^{1v}, apart from presenting the mutation

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TABLE 1. Differences in the sequence of nucleotides between alleles O^1 and O^{1v}

	Exon II	Exon IV		Exon V	Exon VI		Exon VII			
Allele	106[a]	188[a]	189[a]	220[a]	261[a]	297[a]	646[a]	681[a]	771[a]	829[a]
O^1	G	G	C	C	del	A	T	G	C	G
O^{1v}	T	A	T	T	del	G	A	A	T	A

Modified from Olsson et al., 1998.
[a] Position of the nucleotide in the codifying region of the gene.
del, deletion.

which defines O alleles, displays other nine nucleotidic substitutions along its sequence, the majority of them appearing in exon 7 (Olsson and Chester, 1996) (Table 1). This allele would be present in both European descent and Amerindians, with the highest frequencies being found in the latter. In Amazonian aborigines frequencies of 0.9 and 0.65 have been described for allele O^{1v} (Olsson et al., 1998; Barjas-Castro et al., 2003), and on the other hand, in Aymara aborigines from Bolivia it reaches a frequency of 0.60. Frequencies slightly below, but equally significant, were found in aborigines from Ecuador and Bolivia (Roubinet et al., 2001). However, in European descent populations a lower frequency of around 0.40 has been observed (Olsson et al., 1998). A variant allele of O^{1v} with a G542A mutation has been described. This new allele was found in 43% of the indigenous population of Brazil and in a 4% of European descent individuals (Olsson et al., 1998). In general, so far over 70 alleles of the ABO blood group have been described and molecularly defined. These approximations have included methodologies as diverse as PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism), SSCP, ASP, sequencing, and others. The variation exhibited by allele ABO*O is not exclusive to it. Molecular variants have also been described for alleles ABO*A and ABO*B (Yamamoto et al., 1992; Olsson et al., 1998, 2001) opening up interesting future perspectives for research in the fields of molecular population genetics for the ABO blood group system.

The specific objective of the present report is to study the alleles O^1, O^{1v}, and the mutation G542A of ABO blood group in three Chilean populations, 1) indigenous Aymara residents in the first region, 2) indigenous Huilliche residents in the tenth region, and 3) a sample of Santiago consisting of blood donors of the Roberto del Río Hospital. The purpose of this study was to identify Amerind admixture markers and to contribute to the discussion concerning the origin of Andean aboriginal populations.

MATERIALS AND METHODS

Eighty-four individuals of Aymara origin from the Arica highlands (17°S, 70°W), 75 individuals of Huilliche origin from San Juan de la Costa (40° 45′S, 73° 19′S), and finally, 82 individuals from the city of Santiago were included in this study. All individuals participating in this study had blood group O. The determination of alleles O^1 and O^{1v} was carried out on DNA samples obtained from peripheral blood. Blood samples from the aboriginal Aymara and Huilliche populations were obtained over a decade ago within the framework of bioanthropological projects pursuing similar objectives. The Santiago sample was obtained from blood donors of the Roberto del Río Hospital, Santiago northern area, who authorized drawing and use of a sample by signing a document of informed consent in which, as is usual, the research to be undertaken was clearly explained, was anonymous and did not pursue profit.

TABLE 2. Location of the polymorphisms studied, sizes of the fragments obtained after amplification by PCR, and digestion with the respective endonuclease

Exon	Polymorphism	Fragment size PCR	Restriction enzyme	Fragments obtained
IV	G188A; C189T	148 pb	BstUI	**65/83**
VI	G261−	187 pb	KpnI	**54/133**
VII	G542A	229 pb	NheI	**184/45**
VII	T646A	373 pb	MboI	68/**72**+**24(96)**/209
VII	C771T	373 pb	DdeI	97/**169**+**107(276)**

The boldface numbers correspond to the diagnostic fragments for the determination of the molecular alleles included in the study.

The blood group of each individual was determined both by serologic and molecular methods. Genomic DNA was extracted from peripheral blood lymphocytes (buffy coat) according to the protocol described by Lahiri and Nurnberger (1991).

The polymorphisms allowing for the molecular differentiation of the different alleles of the ABO blood group were those corresponding to exons IV, VI, and VII (Table 2). The PCR technique amplification protocols and the pairs of dividing oligonucleotides used were taken from several previous studies, with some modifications (Yamamoto et al., 1990; Ogasawara et al., 1996; Olsson and Chester, 1996; Olsson et al., 1998; Yip, 2002). These included the amplification of the fragments of the gene codifying for the ABO blood group, where the polymorphisms to be studied are found. The amplifications were then submitted to enzymatic digestions with specific restriction endonucleases which allowed for the characterization of the different alleles presented in this study. Subsequently, the genotypic and gene frequencies were obtained directly by counting.

RESULTS

All individuals included in the present study were homozygotes for the G261− deletion, which defines allele O^1. Table 3 summarizes published gene frequencies for O^1 and O^{1v} alleles in Southern Amerinds. Results obtained in this study indicate that allele O^{1v} shows frequencies of 0.65 and 0.81 in Aymaras and Huilliches, whereas the city of Santiago population presents a frequency 0.60.

The mutation G542A was found in a heterozygote state in individuals of genotypes O^1/O^{1v} and O^{1v}/O^{1v}. In agreement with previous studies it was not found in individuals of genotype O^1/O^1 (Table 4).

The frequencies of allele $O^{1v(G542A)}$ were of 0.119, 0.113, and 0.079 in Aymaras, Huilliches, and Santiago populations, respectively. It is worthy of notice that the

TABLE 3. Gene frequencies of O alleles of the ABO blood group system defined molecularly in South American Amerinds

Population	O^1	O^{1v}	Other O alleles	n[a]	References
Arara (Brazil)	0.03	0.97	–	15	Olsson et al. (1998)
Aymara (Bolivia)	0.27	0.60	0.13	252	Roubinet et al. (2001)
Aymara (Chile)	0.35	0.65	–	168	This study
Cayapa (Ecuador)	0.50	0.39	0.11	148	Roubinet et al. (2001)
Chango (Chile	0.39	0.61	–	80	Henríquez et al. (2004)
Huilliche (Chile)	0.19	0.81	–	150	This study
Kayapo (Brazil)	0.16	0.84	–	16	Olsson et al. (1998)
Parakaña (Brazil)	0.35	0.65	–	124	Barjas-Castro et al. (2003)
Yanomama (Brazil	0.09	0.91	–	17	Olsson et al. (1998)

[a] n indicates the number of chromosomes analyzed.

TABLE 4. Association between mutation G542A and alleles O^1 and O^{1v}

	Number of individuals according to mutation G542A, to the homozygote or heterozygote state		
Genotype	GG	GA	AA
Aymara			
O^1/O^1	8	0	0
O^1/O^{1v}	35	8	0
O^{1v}/O^{1v}	21	12	0
Huilliche			
O^1/O^1	4	0	0
O^1/O^{1v}	15	5	0
O^{1v}/O^{1v}	39	12	0
Santiago			
O^1/O^1	18	0	0
O^1/O^{1v}	28	3	0
O^{1v}/O^{1v}	23	10	0

TABLE 5. Percentages of $O^{1v(G542A)}$ allele in various Amerindian populations

Population	Country	$O^{1v(G542A)}$ allele frequencies	References
Arara	Brazil	0.456	Olsson et al. (1998)
Aymara	Chile	0.119	This study
Cayapa	Ecuador	0.041	Roubinet et al. (2001)
Huilliche	Chile	0.113	This study
Kayapo	Brazil	0.395	Olsson et al. (1998)
Parakaña	Brazil	0.221	Barjas-Castro et al. (2003)
Yanomama	Brazil	0.428	Olsson et al. (1998)

frequencies of the mutant allele G542A in the indigenous Chilean Aymara and Huilliche populations are lower than the frequency of 0.43 and 0.22 described for Amerindian populations of Brazil (Olsson et al.,1998; Barjas-Castro et al., 2003). In this context, allele $O^{1v(G542A)}$ frequencies in Southern Amerinds are illustrated in Table 5. The average frequency is 0.25, whereas North Europeans exhibit significantly lower values (~0.04). Furthermore, Asians present frequencies of 0.50 whereas French and Spanish Basques, as well as Africans, show values close to zero (Olsson et al., 1998; Roubinet et al., 2001).

DISCUSSION

For the ABO blood group system O is the most frequent allele in all populations, and particularly in South American Amerinds. Before Columbus's arrival, Central and South America was isolated from Europe and may have had infectious diseases not shared by other populations, as for example syphilis and related treponematoses (Rothschild, 2005). According to Vogel and Helmbold (1972) who analyzed data on blood groups and syphilis (collected before penicillin therapy was available), there was no association between risk of new syphilis infection and ABO blood groups after neosalvarsan treatment. However, O individuals had a better chance to become seronegative than did those with other blood groups. Furthermore, tertiary syphilis was less frequent among blood group O subjects. If syphilis influenced reproduction through infection and death of the fetus, the high frequency of O could be due to selection.

Recently, however, the observation of a combination of both proliferative and destructive processes, which is pathognomonic for syphilis, in two of four pre-Columbian skeletons from the site Hull Magistrater Court in England, is evidence against an American origin of the disease and consequently also against selection (von Hunnius et al., in press). Alternative explanations are obviously selection associated to other infectious diseases or fixation or the O allele by founder effect.

It is, without doubt, interesting to find out whether in these populations allele ABO*O shows variants or is homogeneous for only one molecular type. Our findings agree with the results of other authors, as respects the greater frequency of allele O^{1v} in indigenous South American populations (Table 3). Thus, allele O^{1v} in the Huilliche population presents a high frequency (0.81), approaching that described for some native Brazilian populations (0.91) (Olsson et al., 1998). The distribution of O^{1v} is somewhat heterogenous among Southern Amerinds, ranging from 0.39 to 0.91, but its frequency is significantly higher than in European and Africans. The indigenous Aymara studied by us show a frequency of allele O^{1v} of 0.61 similar to the results obtained by Roubinet et al. (2001) for Bolivian Aymara. The difference between Chilean Aymara and Huilliche was found to be statistically significant ($P \leq 0.001$; Table 3). This finding may, in theory, indicate that the Amerindians of northern Chile (Aymara) have a different origin than the southern groups (Huilliche). Archeological, craniometrical, and protein marker data suggest, however, that Chile's territory was peopled from north to south (Rothhammer et al., 1986; Llop, 1996), giving support to the alternative hypothesis that the heterogenous distribution of alleles O^1 and O^{1v} in Chile is probably the result of a genetic microdifferentiation that occurred during the Paleoindian peopling, that was without doubt a millenary process. In fact, as can be inferred from the observed mtDNA Amerindian haplogroup distribution for different Chilean population located geographically from

latitude 17° to latitude 55°S, haplogroups A and B decrease from north to south, whereas haplogroups C and D increase, giving rise to a cline that backs up the hypothesis of a north to south peopling of Chile's territory (Moraga et al., 2000, 2005; Rocco et al., 2002; García et al., in press).

The fact that the population of Santiago exhibit O^{1v} frequencies which are similar to the frequencies obtained in Aymara (Table 4) can be explained because the Santiago population originated from the admixture of two stem groups, European conquerors and Picunche Indians related to the Araucanian Confederation whose gene frequencies were more similar to the southern Amerindian groups. If we assume that the gene frequency of the Huilliche is representative of these groups and that the frequency of O^{1v} in Spanish immigrants was 0.35 (average between Basques and North Europeans), we can compute the Amerindian admixture of the sample of Santiago using Bernstein's (1931) formula obtaining 46% admixture. This quantity is in close agreement with estimates obtained using other protein marker data (Rothhammer, 1983).

It has been stated that the G542A mutation is physiologically irrelevant, as the inactivating mutation in the O^1 gene is the deletion at nucleotide 261G, but it constitutes a polymorphic marker that can be used to distinguish among Amerindian populations which are otherwise similar for ABO blood groups. The very low frequency of G542A in Africans and Europeans, which can be explained most probably by ancient admixture, makes it possible to use this marker to estimate admixture.

CONCLUSIONS

Our findings are in agreement with the results of other authors which indicate that the O^{1v} allele exhibits a higher frequency in South Amerindians. Furthermore, the heterogenous distribution of O^{1v} in Chile is probably the result of a genetic microdifferentiation that occurred during the Paleoindian peopling some 12,000 years ago. Finally, allele G542A has a relatively low frequency in Chile and can be used successfully as an Amerind admixture marker.

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