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Genetic structure of three Native Mexican communities based on mtDNA haplogroups, and ABO and Rh blood group systems

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ABSTRACT

Objective. The goals of this population genetics study were to describe mtDNA haplogroups and ABO and Rh blood group systems of 3 Native Mexican populations, to determine their genetic variability, and to compare their haplogroups with those of 13 Native Mexican populations previously reported. **Material and methods.** The three communities under analysis were a Tepehua-speaking community from Huehuetla (Hidalgo state), an Otomi-speaking community from San Antonio el Grande (Hidalgo state), and a Zapotec-speaking community from Juchitán (Oaxaca state). Every subject studied in each community had four grandparents who were born in the same community and spoke the same language. The four Amerindian mtDNA haplogroups (A, B, C and D) were studied by restriction analysis and gel electrophoresis. **Results.** Regarding the blood groups, the O group was the most frequent in the three populations (97.2, 94.7, and 86.2%, respectively), as well as the Rh+ group (100, 100, 84%). The three populations analyzed were in Hardy-Weinberg equilibrium. In respect to the mtDNA haplogroups, A, B, C and D, their percentage was 33.3, 36.1, 13.9 and 5.6 % in Huehuetla; 39.5, 13.2, 39.5 and 2.6 % in San Antonio el Grande, and 55.3, 21.0, 7.9 and 5.2 % in Juchitán. Between 5 and 11% of the haplogroups were of non-Amerindian origin, probably due to admixture with Caucasian and African populations, as has been reported in the past. No statistically-significant differences were found among the three populations studied or between them and 13 previously reported Native Mexican populations.

Estructura genética de tres comunidades indígenas mexicanas basada en mtADN, haplogrupos, ABO y Rh del grupo sanguíneo

RESUMEN

Objetivo. Las metas de este estudio de genética de poblaciones fueron las de describir los haplogrupos de mtDNA y los sistemas de grupos sanguíneos ABO y Rh en tres poblaciones indígenas mexicanas, establecer la variabilidad genética entre estas tres poblaciones indígenas mexicanas y comparar sus haplogrupos con los de otras 13 poblaciones indígenas, cuyo perfil de mtDNA ya se conoce. Material y métodos. Se estudió a la comunidad de Huehuetla en el estado de Hidalgo, de lengua tepehua; la comunidad de San Antonio el Grande en el estado de Hidalgo, de habla otomí; y la comunidad de Juchitán en el estado de Oaxaca, de habla zapoteca (estas dos últimas lenguas pertenecen a la familia lingüística otomangue). Resultados. El grupo sanguíneo O fue el más frecuente en las tres poblaciones (94.7, 97.2 y 86.2%, respectivamente), así como el grupo Rh+ (frecuencia de 1, en los dos primeros, y de 0.84 en el tercero). En las tres poblaciones, las frecuencias estuvieron en equilibrio de Hardy-Weinberg. El porcentaje de haplogrupos A, B, C y D fue de 33.3, 36.1, 13.9 y 5.6% en Huehuetla; de 39.5, 13.2, 39.5 y 2.6% en San Antonio el Grande; de 55.3, 21.0, 7.9 y 5.2% en Juchitán. Además, se observó que de 5 a 11% de los haplogrupos no correspondieron a ninguno de los ya mencionados, y podrían deberse a mestizaje con caucásicos y africanos, como lo han reportado otros estudios. No se observaron diferencias estadísticamente significativas a ni-

vel de haplogrupos de mtDNA entre las tres poblaciones estudiadas, ni tampoco al compararlas con otros 13 grupos mexicanos.

Key words. mtDNA. ABO blood group. Native Mexican populations.

Palabras clave. mtDNA. Grupos sanguíneos. Poblaciones indígenas mexicanas.

INTRODUCTION

The current Mexican territory has a great diversity of Native groups, each one with its own cultural, linguistic and geographical identity. This region has seen successive waves of colonization, starting 15,000 years ago, with the arrival of Asian groups, and ending in the XVI Century, with the arrival of Europeans, mainly from Spain, and Africans, in the context of the slave trade.[1-4] The official language of Mexico is Spanish; its present indigenous population comprises around 10 million people, of whom, 6,011,202, aged 5 years or older, speak a native Mexican language, mainly Nahuatl, Maya or Mixtec.[5]

Native languages have been considered a criterion for characterizing human populations[6] and, from a biological perspective, the ABO and Rh blood group systems and mitochondrial DNA (mtDNA) have also been employed for this purpose. Among the former, the O+ group is prevalent in Native Mexicans.[7,8] As for the mtDNA, which is inherited only from the mother, a number of polymorphisms have been identified, including base changes and deletions. The combination of these polymorphisms defines a haplotype, of which, the A, B, C, and D are found in Native American populations.[9-12]

Few Native Mexican population mtDNA investigations have incorporated information on the linguistic family of the subjects studied, even though this may provide a more precise characterization of the populations.[13-15] The present study included two communities, San Antonio el Grande and Huehuetla in Hidalgo state, that differ in their language (Otomi and Tepehua, respectively), and a third population, Juchitán in Oaxaca state (geographically distant from Hidalgo), where Zapotec (belonging to the Otomanguean linguistic family, as does the Otomi from San Antonio el Grande) is spoken.[16,17]

OBJECTIVE

The goals of this population genetics study were: first, to determine the gene frequencies of the blood groups ABO and Rh, and, second, to describe the mtDNA haplogroup frequencies of the three Native Mexican populations.

MATERIAL AND METHODS

Whole-blood samples of 112 non-related Native Mexicans from three populations were collected in tubes with EDTA, and analyzed at the Department of Genetics at Hospital Infantil de México Federico Gómez, in Mexico City. The study was approved by the institute's Research and Ethics Committees. All individuals were instructed on the nature of the study, and those willing to participate were requested to sign a consent form. Participants were included only if they were healthy and if they and their four grand-parents had been born in the same community. Samples collected were 38 from San Antonio el Grande, 36 from Huehuetla and 38 from Juchitán. The former two are small towns in Hidalgo State, while Juchitán is a city of approximately 200,000 inhabitants in the state of Oaxaca. The distance between San Antonio and Huehuetla is 4.33 km (Figure 1).

Blood group determination

ABO and Rh blood groups were determined using antisera and a commercial procedure, following the manufacturer's instructions (LICON).

DNA extraction

Total DNA was extracted from whole-blood using a commercial kit (Gentra), following the supplier's instructions. The DNA was quantified, and its integrity verified, by agarose gel electrophoresis.

mtDNA haplogroup analysis

mtDNA was amplified by PCR, as described by Moraga, *et al.*[18] Amplifications were performed in a volume of 20 μL, with 200 ng of total DNA, and a final concentration of 20 pmol of each primer, 0.2 mM dNTPs and 1.5 mM $MgCl_2$, with 2 U of *Taq* polymerase (Invitrogen). The PCR profile comprised an initial denaturation step at 95 °C for 2 min, followed by 30 cycles at 95 °C (1 min), 55 °C (1 min) and 72 °C (1 min), with a final extension step at 72 °C for 5 min.

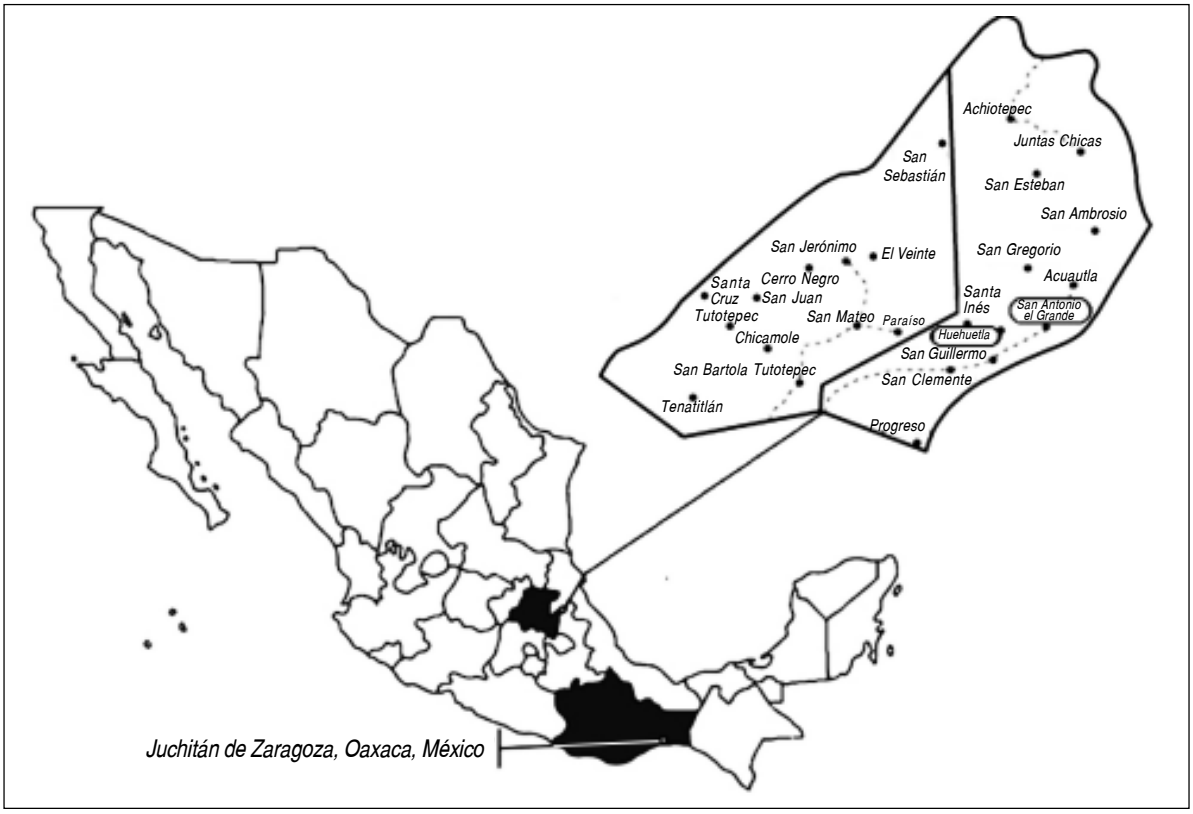

Figure 1. Geographic location of the studied populations.

Restriction analysis

All samples were screened for the presence of polymorphic sites at positions 663, 13259 and 5176.[19] These positions correspond to *Hae*III, *Hinc*II and *Alu*I restriction sites, respectively. The 9 bp indel, starting at nucleotide 8271 in the region of the COII/ $tRNA^{lys}$ gene, was also analyzed. Restriction analyses of the PCR products were done in a 10 μl volume, with 5 μl of the PCR product and 1 U of enzyme (Invitrogen), incubating at 37 °C for 3. Results were analyzed in 2% agarose gels. mtDNA haplogroup B is characterized by a 9 bp deletion. To visualize this, PCR products were separated in 8% polyacrylamide gels (PAGE). Agarose and PAGE gels were stained with ethidium bromide to visualize the PCR fragments. Haplogroups were identified based on the restriction sites, as reported by Moraga, *et al.*[20]

Statistical analysis

Allele frequencies for ABO and Rh groups were computed by the maximum-likelihood method.[21] Hardy-Weinberg Equilibrium (HWE) was tested by the likelihood ratio,[22] exact[23] and χ^2 for total heterozygosity test. A significance level of 0.05 was used, with Bonferroni's correction for multiple testing.[20] Calculations were carried out using a permutation program described by Chakraborty.[24,25] For mtDNA, genetic distances among 16 Mexican Natives populations were computed by Nei's standard genetic distance,[26] and their standard errors (SE) by Neil and Roychoudhury's method.[27] To determine the significance of the genetic distances among the different populations for the mtDNA data, pairwise comparisons were done for the frequencies, using the χ^2 statistic.[27]

RESULTS

Blood group determination

The results for the ABO and Rh blood groups are shown in table 1. Allele frequencies for both blood groups were in Hardy-Weinberg equilibrium. We observed a predominance of blood group O, with an average of 92.71%, in the three populations.

PCR-RFLPs

Haplogroup frequencies were determined as described in Moraga, *et al.*[20] After RFLP analysis and polyacrylamide electrophoresis (for haplogroup B identification) had been run, each individual was assigned to one of 4 mtDNA haplogroups (Table 2). The haplogroup of 10 individuals did not correspond to any of those characteristic of Native American people (A, B, C or D). These samples were not included in the statistical analysis.

Genetic distance

Standard genetic distances, average heterozygosity and χ^2 values among 16 Mexican communities are shown in tables 3 and 4. The tables showing Nei's standard genetic distances were computed for all pairs of populations: 3 from the present work, Huehuetla, San Antonio and Juchitan, and 13 previously reported.[15] Mixteca-Alta, Mixteca-Baja, Otomi, Purepecha, Tzeltal, Tarahumara, Huichol, Nh-Atocpan, Nh-Xochimilco, Nh-Zitlala, Nh-Chilacachapa, Nh-Ixhuatlancillo, and Nh-Necoxtla (excepting Nh-Coyolillo). Also shown are the standard error of the genetic distance (DS) and the pairwise χ^2 matrix for all loci. No statistically significant differences were observed.

DISCUSSION

The present study was aimed at describing three different biological marker frequencies in three different communities. The blood group frequency analysis indicated a predominance of blood groups O and Rh+ in the three populations, in agreement with previous reports.[7,8] A predominance of blood group O was observed, with an average of 92.71% in the three populations, which was similar to what has been described by Lisker[7] in isolated indigenous populations; this has been ascribed to possible bottlenecks or founder effects during the colonization of the American continent, approximately 35,000 years ago.[28] However, a few individuals, in each one of the three populations studied here, carried blood groups A and B (Table 1), with Juchitan being the population with the highest A frequency. These data are interesting as they seem to indicate that even groups considered to be indigenous and inhabiting geographically distant locations, are not totally isolated genetically, as may be thought to be the case for small communities in this country.

The mtDNA haplogroup frequencies of three populations were not statistically different. The number of individuals harboring mtDNA haplogroup B was smallest in San Antonio el Grande, whereas the highest recorded number with this haplogroup was

Table 1. Allelic frequencies of ABO and Rh blood groups in the studied populations.

ABO system	Allele	Huehuetla	San Antonio	Juchitán
	A	0.0140	0.0267	0.0970
	B	0.0140	0.0267	0.0404
	O	0.9720	0.9466	0.8626
	χ^2 HWE	0.014	0.057	0.344
Rh system	+ (D)	36	38	37
	- (d)	0	0	1
		1.0000	1.0000	0.8378
		0.0000	0.0000	0.1622

Table 2. Frequency of mtDNA haplogroup and number of individuals in the studied populations.

	A	B	C	D	Other	Total
Huehuetla	0.3333 (n = 12)	0.3611 (n = 13)	0.1389 (n = 5)	0.0556 (n = 2)	0.1111 (n = 4)	1.0000 (n = 36)
San Antonio el Grande	0.3947 (n = 15)	0.1316 (n = 5)	0.3947 (n = 15)	0.0263 (n = 1)	0.0527 (n = 2)	0.9990 (n = 38)
Juchitán	0.5526 (n = 21)	0.2105 (n = 8)	0.0789 (n = 3)	0.0526 (n = 2)	0.1054 (n = 4)	1.0000 (n = 38)

n: number of individuals.

Table 3. Standard genetic distances, and average heterozygosity among 16 Mexican indigenous populations.

Standard genetic distance (DS) matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	45.08														
3	39.25	19.14													
4	51.90	1.69	-5.77												
5	59.23	3.80	-3.84	-4.22											
6	58.58	-4.36	48.12	24.95	21.17										
7	74.82	-5.66	24.48	6.21	1.95	0.93									
8	75.65	-1.29	18.23	-3.57	8.96	15.99	2.60								
9	41.82	2.66	-6.99	-7.30	-6.93	22.62	6.63	6.12							
10	21.93	-9.03	12.80	2.13	5.57	0.00	1.91	5.08	0.87						
11	37.69	86.28	1.11	40.79	38.38	127.52	99.11	93.14	30.36	64.00					
12	55.15	131.85	32.48	75.09	86.41	182.92	154.74	131.11	69.14	102.66	-13.81				
13	95.41	41.96	-8.24	6.56	-2.61	74.39	33.94	35.15	3.85	42.55	11.08	55.29			
14	32.22	-23.45	17.25	-5.40	1.12	-17.97	-14.49	-13.09	-2.50	-20.98	91.45	134.18	47.76		
15	35.68	-10.86	20.64	2.78	9.01	-2.91	-1.15	-0.56	4.70	-10.45	85.40	125.53	50.39	-24.44	
16	29.70	-6.79	31.07	10.63	21.63	0.08	7.59	4.87	13.64	-8.45	96.79	131.68	70.82	-23.22	-10.45

S.E. of standard genetic distance (DS)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	65.74														
3	60.02	26.83													
4	79.35	1.60	8.74												
5	87.74	5.33	6.73	6.79											
6	79.96	6.94	64.13	31.15	27.46										
7	106.76	8.14	31.80	6.17	2.04	1.10									
8	114.18	1.95	26.42	5.61	12.74	20.02	2.60								
9	63.09	3.33	10.65	10.86	10.31	28.42	7.36	8.74							
10	32.38	13.01	18.65	2.94	7.85	2.33	1.30	7.29	1.25						
11	57.41	129.82	1.65	62.61	56.22	185.31	144.45	142.92	45.87	97.41					
12	84.78	203.67	50.08	117.30	130.83	275.59	233.87	205.35	106.69	159.57	20.72				
13	143.85	60.00	13.24	8.78	3.67	102.60	46.54	50.61	5.12	61.79	15.23	81.80			
14	47.40	32.84	24.91	8.28	1.57	25.97	20.75	18.59	3.78	29.89	140.10	210.40	69.21		
15	52.52	15.31	29.90	3.62	12.82	5.68	2.46	0.77	6.64	14.98	130.20	195.63	73.05	34.45	
16	43.71	9.67	45.91	15.29	31.02	1.79	9.49	6.99	19.81	12.14	148.96	206.49	103.97	32.83	14.85

found in Huehuetla. These data are particularly interesting as it has been reported that mtDNA haplogroup B is more frequent the further South on the American continent this analysis is carried out. However, San Antonio el Grande does not lie further South than Huehuetla or, indeed, than Juchitan[29,30] (Figure 1). The Huehuetla and the San Antonio el Grande communities from this study live in Hidalgo state and the languages spoken there, Otomi and Tepehua, respectively, belong to two different families; on the other hand, the geographically distant community in Juchitan speaks Zapotec, which is a tonal language belonging to the same linguistic family as Otomi. However, and contrary to what could have been expected, our results did not show evidence of a closer relationship among communities that were geographically closer nor among those separated by a larger distance but having languages belonging to the same family.

There was the possibility to further compare the mtDNA haplogroup data from our populations with those from 13 populations previously reported.[15] This analysis did not show any significant differences. Some of the present results can be compared to those provided in a report on Mexican mestizo individuals.[31] Results from the former study are in agreement with those in the latter report since a higher Amerindian ancestry was found among individuals from central regions of Mexico than in those from Mexico City and the coastal areas, due to

Table 4. Chi-square values among 16 Mexican indigenous populations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.5706														
3	0.5323	0.2481													
4	0.7898	0.1286	0.1297												
5	0.7924	0.2319	0.2262	0.2490											
6	0.6691	0.1609	0.5619	0.4584	0.2379										
7	1.0203	0.1444	0.4516	0.2711	0.1742	0.1960									
8	1.2729	0.3244	0.5946	0.2023	0.5410	0.6002	0.2907								
9	0.5758	0.0952	0.0426	0.0848	0.1058	0.3003	0.2106	0.3822							
10	0.3217	0.0494	0.1977	0.1968	0.2829	0.1960	0.3041	0.4964	0.0958						
11	0.6468	0.9838	0.3436	0.8586	0.6546	1.1398	1.4709	1.9809	0.5605	0.7247					
12	0.7114	1.2456	0.5023	0.8606	1.0807	1.6205	1.5162	1.4162	0.7437	0.9880	0.1894				
13	1.1186	0.5186	0.2173	0.3101	0.0998	0.6938	0.3930	0.7708	0.2227	0.5994	0.5022	0.8910			
14	0.5208	0.0290	0.3413	0.1552	0.5669	0.4565	0.3271	0.5667	0.1795	0.0703	1.1991	1.7430	0.7317		
15	0.4895	0.0229	0.2657	0.1388	0.3392	0.2184	0.2682	0.3489	0.1310	0.0340	0.9762	1.1674	0.6667	0.0092	
16	0.4387	0.1070	0.3921	0.2398	0.5310	0.3094	0.4527	0.4414	0.2530	0.0775	1.1146	1.2113	0.9584	0.0379	0.0336

Pairwise Chi square Matrix for all loci: Degrees of Freedom = 3.

more European female ancestors having settled in the country's capital.[31] Therefore, it may be proposed that the observed differences could be due to the heterogeneous settlement of Native communities from the central area of Mexico. In this regard, the present results could be explained by differences in historical migration routes within these regions, or a low/high interaction with neighboring communities.

The present study describes three Native Mexican populations belonging to two different linguistic families using three biological markers. A particular aspect that should be further studied is the one regarding the 10 individuals that could not be characterized using the four more frequent Amerindian mtDNA haplogroups. Other haplogroups could be taken into consideration, such as haplogroup X, which has been reported in Amerindian populations, or, indeed, haplogroups H, J, K, T U and V of European origin, which are found in Mexican populations.[28,31] Further investigations analyzing the D loop mtDNA region in these communities could give additional insight into the genetic relationships among different groups of Native Mexicans.

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