TECHNICAL NOTE

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Quechua Amerindian Population Characterized by HLA-DQα, YNZ22, 3′APO B, HUMTH01, and HUMVWA31A polymorphisms

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ABSTRACT: Allele and genotype frequencies of DNA polymorphisms were determined in a population sample of Quechua (n = 113) using the polymerase chain reaction (PCR). We report data on the frequencies of HLA-DQα, YNZ22, 3′ApoB, HUMTH01 and HUMVWA31A alleles and the distribution of the different genotypes. No significant deviations between observed and expected numbers were found, thus assuming the Hardy-Weinberg equilibrium.

KEYWORDS: forensic genetics, HLA-DQα, YNZ22 (D17S5), 3′ApoB, HUMTH01, HUMVWA31A, Quechua population

The amplification of DNA sequences by means of polymerase chain reaction (PCR) has provided a number of rapid approaches to determine DNA polymorphisms. Although many populations have been studied using DNA polymorphisms, to date there is a complete lack of tetrameric STRs data of autochthonous South American Indians. This paper presents data on the frequencies of HLA-DQα, YNZ22, 3′ApoB, HUMTH01, and HUMVWA31A alleles and the distribution of the different genotypes in a sample of Quechua population.

The Quechua is the language spoken today by the descendants of the Incas, as well as by the tribes they conquered, in Peru, Ecuador, Colombia, and Bolivia. The center of Quechua is in the highlands of Peru, Bolivia, and Ecuador, but it has spread to the highland areas of southern Colombia, northern Chile and Argentina, the west coast of Peru, and the lowlands of Peru and Bolivia on the east slope of the Andes Mountains. At present the Quechua population is represented by 5 to 7 millions inhabitants (1). The Quechuas studied belong to the province of Dalence, Department of Oruro (Bolivia).

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Materials and Methods

Preparation of DNA

The biological samples analyzed consist of two hair-pulls with bulb from 113 unrelated individuals (both sexes) from the Quechua ethnic. DNA was extracted with Chelex™ 100 using the method described by Walsh et al. (2).

PCR Amplification and Typing

The HLA-DQα alleles were detected using the Perkin-Elmer AmpliType™ kit was used. This procedure detects the allelic sequence variation in the locus by hybridization of biotinylated primers PCR product with immobilized allele-specific oligonucleotide probes (ASO), using a "reverse dot-blot" procedure.

YNZ22 and 3′ApoB are Minisatellite VNTR polymorphisms. PCR amplification of 3′ApoB and YNZ22 were accomplished by the methods described by Boerwinkle et al. (3) and Batanian et al. (4), and Walsh et al. (5), respectively, with some modifications introduced by us and described previously for Catalonian (NE Spain) sample studies (6,7). Detection of the different alleles, from PCR-amplification products, were carried out by ethidium bromide 15 × 20 agarose gel electrophoresis (2% Seakem GTG-FMC™ agarose for YNZ22, and 3% NuSieve 3:1 FMC™ agarose for 3′ApoB), along with allelic ladders of a cocktail sample.

HUMTH01 and HUMVWA31A are tetrameric short tandem repeats (STRs), or Microsatellite VNTRs. PCR amplification was achieved using the primers described by Edwards et al. (8) and Kimpton et al. (9) respectively. Forward primers were labelled with fluorescein amidite in 5′ position. The reaction assay and the amplification conditions were carried out as described by Wiegand et al. (10) and Moller et al. (11) respectively. Separation of alleles was done on 6% (w/v acrylamide/bisacrilamide) polyacrylamide denaturing high-performance DNA sequencing gels (Ready Mix Gel ALF grade, Pharmacia) using Automated Laser Fluorescent (ALF) DNA sequencer (Pharmacia). Fluorescent labelled standard markers and a cocktail sample containing all observed alleles was used as an allelic ladder.

TABLE 1—*Genotype and allele frequencies distribution at the HLADQα locus in a sample of 108 Quechua.*

Allele							Frequency
	1.1	1.2	1.3	2	3	4	
1.1	—	—	—	—	1	2	0.014
1.2		—	—	—	—	1	0.005
1.3			—	—	1	1	0.009
2				—	1	—	0.005
3					20	61	0.481
4						20	0.486

Exact Test: P = 0.2135.

Except HLA-DQα, for the rest of loci we used a standard nomenclature for DNA fragments based on the number of repeats. All PCR amplifications were performed together with negative and positive control samples.

Statistical Analysis

The frequency of each allele for each polymorphism was calculated using the gene count method. Sample sizes vary in the different systems (HLA-DQα, HUMTH01, HUMVWA31A, n = 108; YNZ22, n = 103; 3′ APO B, n = 107) due to missing data for some individuals. Possible divergence from Hardy-Weinberg equilibrium (HWE) was determined by calculating the exact test proposed by Guo and Thompson (12). The genotype linkage disequilibrium was calculated using the updated version of GENEPOP (13). From a forensic point of view, the power discrimination (PD) (14) and the "a priori" chance exclusion value (CE) (15) were calculated.

Results and Discussion

For the five systems the genotype and allele frequencies in the population analyzed are shown in Tables 1, 2, 3, 4 and 5. The HLA-DQα analysis demonstrated 7 expected genotypes, representing products of 6 alleles. Alleles 3 and 4 present the highest frequencies with a 96.8% of total. YNZ22 polymorphism presents 8 genotypes representing products of 6 alleles, of which allele 4 presents the highest frequency (80%). At the locus 3′Apo B, 20 genotypes were observed corresponding to 8 alleles. The sum of frequencies of alleles 37, 39 and 49 represents the 75.4% of the total. The HUMTH01 system presents 6 genotypes, product of 3 alleles, with the highest frequencies in allele 7 (61%). For HUMVWA31A has been observed 12 genotypes corresponding to 5 alleles. The alleles 16, 17 and 18 represents the 90.9% of the total allelic frequency.

TABLE 2—*Genotype and allele frequency distribution at the YNZ22 locus in a sample of 103 Quechua.*

Allele											Frequency
	1	3	4	5	6	7	8	9	10	11	
1	—	—	2	—	—	—	—	—	—	—	0.009
3		2	10	—	—	—	—	—	1	—	0.073
4			69	7	3	6	—	—	2	1	0.820
5				—	—	—	—	—	—	—	0.034
6						—	—	—	—	—	0.015
7						—	—	—	—	—	0.029
8						—	—	—	—	—	—-—
9							—	—	—	—	—-—
10								—	—	—	0.015
11									—	—	0.005

Exact test: P = 0.6550.

TABLE 3—*Genotype and allele frequency distribution at the 3′ ApoB locus in a sample of 107 Quechua.*

Allele											Frequency
	31	33	35	37	39	41	43	45	47	49	
31	—	—	2	1	—	—	—	1	1	—	0.023
33		—	—	2	—	—	—	—	3	—	0.023
35			12	15	5	1	—	3	5	2	0.266
37				14	5	—	—	2	14	3	0.327
39					2	1	—	—	3	1	0.089
41						—	—	—	—	—	0.009
43							—	—	—	—	—-—
45								2	4	1	0.070
47									2	—	0.159
49										—	0.033

Exact test: P = 0.1507.

TABLE 4—*Genotype and allele frequency distribution at the HUMTH01 locus in a sample of 108 Quechua.*

Allele						Frequency
	6	7	8	9	9.3	
6	4	28	—	—	4	0.185
7		37	2	1	23	0.593
8			—	—	—	0.009
9				—	2	0.014
9.3					7	0.199

Exact test: P = 0.2264.

TABLE 5—*Genotype and allele frequency distribution at the HUMVWA31A locus in a sample of 108 Quechua.*

Allele								Frequency
	14	15	16	17	18	19	20	
14	—	2	1	—	—	—	—	0.014
15	—	5	16	5	2	—	—	0.153
16			10	20	6	3	2	0.319
17				17	10	4	—	0.343
18					3	2	—	0.120
19						—	—	0.042
20							—	0.009

Exact test: P = 0.1165.

For all the polymorphisms analyzed the distribution of the genotypes are in Hardy-Weinberg equilibrium. An inter-class correlation test analysis demonstrated that there is no detectable evidence for correlation between the alleles at any of the pair-wise comparisons of the five loci. The allele frequencies obtained in the Quechua population for all the polymorphisms clearly differ from Caucasoid populations studied by us (6,7,16,17). Amerindian samples have been analyzed only for HUMTH01 and 3′Apo B systems. For HUMTH01, Quechua differ significantly from Eskimos and Mexican-Americans (18). They also differ for 3′Apo B from Pehuenche and Dogrib Indians (19). The PD and CE values observed for the five systems are shown in the Table 6. The observed allelic frequencies and the theoretical "a priori" forensic values (PD, CE) are lower than previously described for European populations (6,7,16), thus these systems seem less informative for Legal Medicine purposes in the Quechua population.

TABLE 6—*Statistical parameters of medico-legal interest for the STR systems HLA-DQα, YNZ22, 3′APO B, HUMTH01, and HUMVWA31A.*

System	H	PD	CE
HLA-DQα	62.96	0.671	0.229
YNZ22	31.07	0.526	0.184
3′APO B	70.09	0.894	0.585
HUMTH01	55.55	0.764	0.325
HUMVWA31A	67.59	0.890	0.511

H: Heterozygosity value.
PD: Power of discrimination.
CE: Chance of exclusion.

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