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Genetic Admixture Estimate in the Uruguayan Population Based on the Loci LDLR, GYPA, HBGG, GC and D7S8

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ABSTRACT We have analyzed the allele and phenotype frequencies in five DNA loci: LDLR, GYPA, HBGG, GC, and D7S8 in a sample from Uruguay. All the loci were in Hardy-Weinberg equilibrium (HWE) except the D7S8 locus. Our genetic admixture estimate showed evidence that the main genetic contribution comes from Europe with a small Amerindian and a minor African contribution with the admixture proportions: 84.1%, 10.4%, and 5.6% respectively. Genetic distances between the Uruguayan sample and several other Latin American populations revealed the closest genetic relationship with the Argentinean capital city, probably because its common history and demographic characteristics.

INTRODUCTION

Studies carried out in Latin American populations have shown that they have been originated by complex scenery of ethnic, cultural, and social relationships, moreover provide a valuable opportunity for population genetic studies (Salzano and Bortolini 2002). With the exception of some Amerindian isolate ethnic groups practically almost all Latin America populations have suffered an intense process of admixture all along in the last five centuries (Sans 2000).

Uruguay has been considered an immigrants country that, unlike other South American countries, has no Amerindian people and a small African contribution (Ribeiro 1969; Wagley, 1971). However, studies on HLA, blood groups, serum proteins, and red cell polymorphisms have documented the importance of the contribution of African and Amerindian people in the actual Uruguayan population (Alvarez et al. 1993; Sans 1997; Sans et al. 1993, 1997).

The Polymarker (PM) is detected by a polymerase chain reaction (PCR) multiplex amplification and typing kit of five specific regions of the genetic loci: low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gamma globin (HBGG), D7S8, and group specific component (GC); its use has proven highly efficient and informative for forensic and population genetics studies (Herin et al. 1994; Budowle et al. 1995; Chakraborty et al. 1995). A number of recent studies carried out in some American populations have provided additional information on polymarker distribution and consequently, useful to analyze relationships with different populations (Bertoni et al. 2003; Buentello-Malo et al. 2003).

Here, we analyzed the allele and genotype frequencies for the PM loci: LDLR, GYPA, HBGG, GC, and D7S8 in a Uruguayan sample. The results are analyzed with two main goals: (1) to estimate the accumulated admixture contribution of the parental populations (i.e., European, African, and Amerindian) to the Uruguayan population; (2) to assess the relationship of the Uruguayan population with other Latin American populations using the available information.

MATERIALS AND METHODS

The sample was collected from 85 healthy, adult, and unrelated consenting individuals of both sexes, born in various regions of Uruguay, and sampled in Montevideo.

Genomic DNA was extracted from fresh whole blood using a previously described method comprising red cell lysis, proteinase K digestion,

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salt extraction, and ethanol precipitation (Miller et al. 1988) with slight modifications. PCR amplification and typing for the systems LDLR, GYPA, HBGG, GC and D7S8 were carried out using the AmpliType® PM PCR Amplification and Typing Kit (Polymarker Multiplex; Applied Biosystems; Foster City, CA) according to the manufacturer's protocol.

The allele frequencies were calculated by the gene count method (Li 1976). Departures from Hardy-Weinberg equilibrium were tested by the chi-square test and by the heterozygote deficiency test (Rousset and Raymond 1995) using the software Genepop (v.3.4; Raymond and Rousset 1995). Unbiased expected heterozygosity for single loci and average heterozygosity were computed according to Nei (1987).

Admixture estimates were calculated by the method described by Elston (1971), using a trihybrid model. The gene frequencies for the parental populations were calculated as an unweighted average from European, African, and Amerindian samples. The parental gene frequencies, and their sources, are listed in the Appendix.

Estimates of Nei´s standard genetic distances (Nei 1972) were computed to compare the Uruguayan sample with other American populations in which the PM allele frequencies were available. The selected populations share common historical patterns, as well as comparable parental contributions regarding its origins (European, Amerindian, African). The available populations were: four African-derived: Costa Rican Afro-Caribbeans (Morales et al. 2001), Haití (Peterson et al. 2000), French Antilles (Peterson et al. 2000), and Brazilian Mulattoes (Soares-Viera et al. 2003); five heterogeneous samples of South American populations: Brazilian Whites (Soares-Viera et al. 2003), Chilean (Jorquera and Budowle 1998), Argentinean (Padula et al. 1996), Colombian (Castillo et al. 1996), and Uruguayan (this paper); three samples from North and Central America: Mexico City (Peterson et al. 2000), Nicaraguan (Morera et al. 2001), Costa Rican (Morales et al. 2001); three admixed Mexican Native Indian populations: Mixteca-Alta, Mixteca-Baja, and Nahuas-Xochimilco (Buentello-Malo et al. 2003). US Blacks (Peterson et al. 2000), and two samples of Hispanics from United States (West and East) (Bertoni et al. 2003) were also included as references. The neighbor-joining method (NJ) was used to display the matrix of pairwise distance (Saitou and Nei 1987). The PHYLIP (v.3.5c; Felsenstein 1993) package of programs was employed to construct phylogenetic trees from the distance matrices. We used the programs GENDIST, SEQBOOT and then run NEIGHBORN. The consensus tree was built with CONSENSUS program.

RESULTS

Table 1 shows the phenotypes, allele frequencies, and unbiased expected heterozygosity of the studied loci. Departures from the Hardy-Weinberg genetic equilibrium at the single locus level were not statistically significant, with the only exception of locus D7S8 in which we also found a significant departure from Hardy-Weinberg expectation when the heterozygote deficiency test was applied ($p = 0.028$). When all the five loci were analyzed with the multi-locus test for the Hardy Weinberg expectation for heterozygote deficit (Rousset and Raymond 1995), no detectable deviation was observed ($p = 0.1237 \pm 0.0045$). Unbiased expected heterozygosity ranged from 0.4595 (D7S8) to 0.6155 (GC).Overall average heterozygosity showed a slightly high level with a value of 0.5125.

Table 2 presents the estimated admixture proportion of European, African and Amerindian genes in our sample. We obtained a strong global presence of 84.1% genes from European, followed by the Amerindian component (10.4%), and a minor African contribution (5.6%). We obtained an equivalent result by using Chakraborty´s identity method (Chakraborty, 1985) (Table 2).

Nei's (1972) pairwise genetic distance matrix is presented in Table 3, showing the relationship of different American populations. The greatest separate the African derived and non-African derived American populations (Table 3). The distances among South American populations were consistently low. Brazilian whites and Chileans show the shortest distance, whereas Colombians and Uruguayans show the highest distance. To visualized the affinity between the studied populations the consensus NJ tree was drawn (Fig.1). The tree shows two main clusters, whereas African derived populations are distinctly separated from the other samples. Uruguay and Argentine show share single cluster and are distintively different from other

Table 1: Phenotypes and allele frequencies at five loci.

System	*Phenotype*	*Observed frequency*	*Expected frequency*	*Chi-square*	*d.f.*	*P*	*HT*[1]
LDLR							
	A-A	14	11.893				
N = 80	A-B	34	38.214				
	B-B	32	29.893	0.986	1	0.321	0.223
GYPA							
	A-A	32	32.929				
N = 85	A-B	42	40.142				
	B-B	11	11.929	0.185	1	0.667	0.745
HBGG							
	A-A	16	15.982				
	A-B	39	38.970				
N = 85	A-C	3	3.065				
	B-B	23	23.172				
	B-C	4	3.686				
	C-C	0	.124	0.154	3	0.985	0.587
D7SB							
	A-A	40	35.473				
N = 85	A-B	30	39.053				
	B-B	15	10.473	4.633	1	0.031*	0.028*
GC1							
	A-A	12	10.473				
	A-B	10	9.586				
N = 85	A-C	26	29.467				
	B-B	2	2.077				
	B-C	13	13.260				
	C-C	22	20.136	0.829	3	0.843	0.276

	Locus				
	LDLR	*GYPA*	*HBGG*	*D7SB*	*GC*
Allele					
A	0.3875	0.6245	0.4353	0.6471	0.3529
B	0.6135	0.3765	0.5235	0.3529	0.1588
C	0.0412	0.4883			
Heterozygosity					
Unbiased	0.4777	0.4723	0.5379	0.4595	0.6155
Observed	0.4250	0.4941	0.5412	0.3529	0.5765

[1]HT: p values heterozygote deficiency test; *p < 0.05

Latin American populations. A second cluster consists in three subclusters: Chile and Brazilian whites integrated one of them, Nicaragua and Mixteca Alta (Mexico) integrated the second one, and the third one includes three Mexican samples, Colombians, admixed population of Costa Rica, and US Hispanics from the West of the country. Interestingly, US Hispanic from the West and from the East was always placed in separate cluster.

DISCUSSION

Our admixture estimates similar to the ones reported for Montevideo (92% European, 7% African, and 1% Amerindian contributions), and intermediate between them and the ones for the northeast of the country (65%, 15%, and 20% respectively) based on blood groups, serum proteins and red cell polymorphisms (Sans et al. 1997). However, African admixture is slightly lower in our estimation. The differences among

Table 2: Admixture estimates in the Uruguayan sample.

Method	*Estimated proportion of parental contribution*		
	European	*African*	*Amerindian*
Elston (1971)	0.841	0.0536	0.104
Chakraborty (1986)[1]	0.870±0.026	0.027± 0.010	0.103±0.030

[1]R^2 = 0.992

Table 3: Nei´s genetic distances.

	(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*	*(9)*	*(10)*	*(11)*	*(12)*	*(13)*	*(14)*	*(15)*	*(16)*	*(17)*
HAITI	0.021																
AFUS	0.007	0.005															
AFROCR	0.036	0.099	0.068														
BRMUL	0.045	0.123	0.085	0.005													
MXA	0.093	0.180	0.140	0.027	0.030												
MXB	0.075	0.176	0.127	0.023	0.011	0.020											
NXO	0.129	0.252	0.196	0.044	0.036	0.013	0.012										
NICA	0.077	0.172	0.126	0.029	0.020	0.013	0.007	0.014									
HUSE	0.075	0.175	0.127	0.013	0.008	0.017	0.006	0.014	0.012								
HUSW	0.093	0.195	0.146	0.021	0.020	0.009	0.013	0.010	0.010	0.006							
MXC	0.111	0.220	0.164	0.034	0.033	0.020	0.025	0.021	0.020	0.012	0.013						
ADMCR	0.109	0.227	0.168	0.031	0.022	0.017	0.010	0.010	0.010	0.007	0.004	0.011					
CHI	0.132	0.267	0.200	0.047	0.028	0.034	0.012	0.015	0.017	0.012	0.017	0.018	0.005				
COL	0.145	0.279	0.215	0.052	0.038	0.022	0.014	0.007	0.015	0.018	0.011	0.026	0.005	0.007			
BRW	0.106	0.225	0.167	0.029	0.015	0.032	0.010	0.018	0.018	0.005	0.015	0.016	0.008	0.004	0.016		
URU	0.095	0.212	0.157	0.032	0.021	0.047	0.015	0.024	0.027	0.010	0.019	0.031	0.017	0.018	0.026	0.011	
ARG	0.100	0.213	0.162	0.025	0.017	0.026	0.010	0.010	0.020	0.005	0.011	0.023	0.012	0.015	0.017	0.008	0.005

1) ANTFR: French Antilles; 2) HAITI; 3) AFUS: US Blacks; 4) AFROCR: Costa Rica Afro-Caribbean; 5) BRMUL: Brazilian Mulattoes; 6) MXA: Mixteca-Alta; 7) MXB: Mixteca-Baja; 8) NXO:Nahuas-Xochimilco; 9) NICA: Nicaragua; 10) HUSE: US Hispanics Eastern; 11) HUSW:US Hispanics Western; 12) MXC: Mexico City; 13) ADMCR: Costa Rica admixed; 14) CHI: Chile; 15) COL: Colombia; 16) BRW: Brazilian Whites; 17) URU:Uruguay; 18)ARG:Argentine;

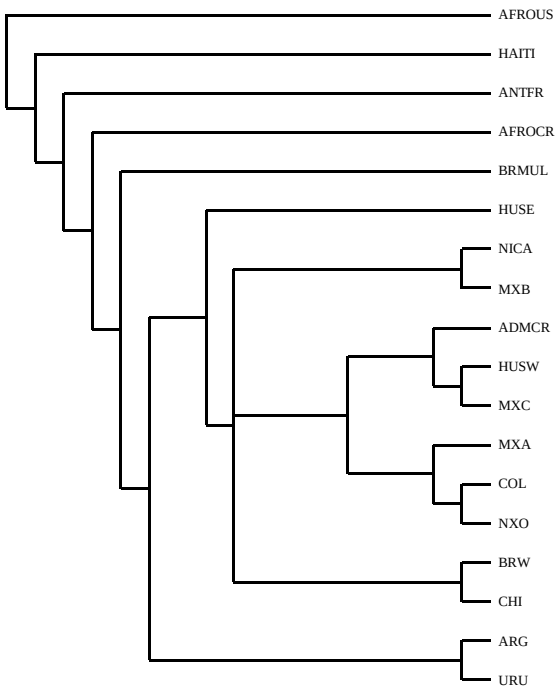

Fig.1. Consensus Neighbor-Joining tree from the Nei´s distances.

AFROUS: US Blacks; ANTFR: French Antilles; AFROCR: Costa Rica Afro-Caribbean; BRMUL: Brazilian Mulattoes; HUSE: US Hispanics Eastern; NICA: Nicaragua; MXB: Mixteca-Baja; ADMCR: Costa Rica admixed; HUSW: US Hispanics Western; MXC: Mexico City; MXA: Mixteca Alta; COL: Colombia; NXO: Nahuas-Xochimilco; BRW: Brazilian Whites; CHI: Chile;
ARG:Argentine; URU: Uruguay.

the samples, admixture estimation methods, parental selection, and genetic markers used, make clear that direct comparisons between these studies warrant caution. Our results suggest that the Uruguayan population present a moderate admixture level with a predominant European parental contribution and a more modest Amerindian and African parental contributions. Then, the data support an admixture trihybrid model modulated by the European migration waves occurred in the last century (Oddone 1966).

Some further comments should be made on the NJ tree in Figure 1. In this tree is evident the relationship between Uruguay and the sample from Argentine (Buenos Aires). Both regions have a common immigrant background with an important Spanish and Italian components (Oddone 1966) Moreover, our results are not surprising if we considering that, the population of the densely populated city of Buenos Aires and surrounding areas, show an African contribution between 3,5 and 7%, and Amerindian contribution between 14 and 33% (López-Camelo et al. 1996, Avena et al. 1999, 2001). In general, the Uruguayan population genetic make up is similar to other admixed urban Latin American populations, with a predominant European-derived component.

The Uruguayan-Argentinean cluster is separated to the other cluster that deserve our attention, formed by Chileans, Brazilian whites, Colombians, Costa Ricans, US Hispanics from the West, and 4 Mexican populations. This cluster denotes the Amerindian contribution, greater in those areas (Sans 2000). US Hispanics from the East is also in this cluster but a little more distant, probably indicating more African contribution (Bertoni et al. 2003) African-derived populations do not clusterize and are beyond the others. Moreover, the results of the frequencies of the alleles studied here clearly demonstrate a significant heterogeneity among the populations of Latin American in agreement with the different regional proportions of the European, Amerindian and African parental contributions to the admixture process for each American country. All the Latin American populations analyzed are far away of having a homogeneous ethnic composition, and none of them is free of gene flow and admixture effects. This study illustrate the genetic heterogeneity present in the Latin American populations, for that reason, in the context of genetic epidemiology, future studies related to disease risk factors will need to address a research design that subdivides the population using genetic parameters (Chakraborty et al. 1999).

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APPENDIX

Parental gene frequencies[1]

Allele	*European*	*African*	*Amerindian*
LDLR*A	0.4634	0.1821	0.634
LDLR*B	0.5636	0.8179	0.366
GYPA*A	0.534	0.5087	0.8239
GYPA*B	0.466	0.4313	0.1761
HBGG*A	0.4845	0.3815	0.2772
HBGG*B	0.503	0.2197	0.7112
HBGG*C	0.0125	0.3988	0.0116
D7S8*A	0.5972	0.6734	0.5678
D7S8*B	0.4026	0.3266	0.4322
GC*A	0.3088	0.0867	0.1486
GC*B	0.1495	0.8439	0.2916
GC*C	0.5417	0.0694	0.5598

[1] Sources of parental populations:
European: Spain, Portugal, and Italy from the compilation of http://www.uni-duelssedorf.de/ WWW/ MedFak /Serology/ polymarker.html.
African: Nigeria, and Zimbabwe from Peterson et al. (2000).
Amerindian: Alaskan Amerindians from Walkinshawn et al. (1996), North American Indians: Navajo, Pueblo, and Sioux from Scholl et al. (1995), Mexican: Nahuas-Guerrero, Tzeltales, Otomies, and Purepecha from Buentello-Malo et al. (2003), and Bribri-Cabecar from Costa Rica (Morales et al. 2001). No data available for PM from South American Indians.