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Nicaraguan population data on LDLR, GYPA, D7S8, HBGG, GC and HLA-DQA1 loci

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Abstract: Nicaraguans have become the most numerous and fastest increasing minority in Costa Rica: at present they represent around 6 % of the total population of the country. We have analyzed the allele and genotype frequencies of six PCR-based genetic markers (LDLR, GYPA, HBGG, D7S8, GC, and HLA-DQA1) in 100 unrelated Nicaraguans living in Costa Rica. All *loci* studied were in Hardy-Weinberg equilibrium. Some statistical parameters of forensic interest were also calculated (h, PD and CE). Allele frequencies of the markers HLA-DQA1 and GYPA were found to be significantly different between the populations of Nicaragua and Costa Rica. Nevertheless, genetic distances showed that Nicaragua is close to other Hispanic-admixed populations like those from Argentina, Chile, Colombia, Costa Rica, and USA Hispanics. The *loci* set was assessed to be useful for paternity testing and individual identification in the Nicaraguan population residing in Costa Rica.

Key words: PCR-based, LDLR, GYPA, HBGG, D7S8, GC, HLA-DQA1, genetic markers, polymorphism, Nicaragua, Costa Rica.

Until a some years ago, the immigrant population formed a very low proportion of the total population of Costa Rica. However, during the last 30 years Nicaraguans in Costa Rica experienced a considerable increase both by immigration and birthrate. Now, at around 6 % of the residing population of the country, they passed from being 1.4 % to becoming the most numerous minority (Schmidt 1979, Chen Mok *et al.* 2000, Anonymous 2001a).

International standards demand the existence of population-specific data that support the biostatistical calculations in cases when an exclusion can not be achieved, as much in the paternity investigations as in the analyses of biological remains of criminological interest

(Carracedo *et al.* 1997, Anonymous 2000, Gómez and Carracedo 2000). In 1997, the Judicial Branch of Costa Rica introduced DNA technology in paternity and forensic testing and, as a result, an extensive study of the distribution of the DNA genetic markers in the Costa Rican population was made possible (Morales-Cordero *et al.* 2001). The official historiography has presumed, since the last decades of the 19th century to the present day, that the people in Costa Rica are ethnically different from their neighbors in Nicaragua (Meléndez Obando 1999, Sandoval García 1999, Acuña Ortega 2001, Dobles 2001). However, to the best of our knowledge, the population genetics of the general Nicaraguan nation remains completely unknown.

Since the influx of Nicaraguans requiring judicial services for biological analyses is not low, and due to the fact that it occasionally concerns cases of penal character, it became clear the necessity of also knowing the gene frequency distribution of those markers in them. That information is of the utmost importance for an adequate administration of justice. The objective of this study was to analyze the genetic particularities of the Nicaraguan population residing in Costa Rica and to create a reference database to contribute to the resolution of civil and penal cases involving persons of that nationality.

MATERIALS AND METHODS

The *loci* LDLR, GYPA, D7S8, HBGG, GC, and HLA-DQA1 were analyzed in a sample of 100 adult, unrelated, volunteer donors of both sexes, originating from different regions of Nicaragua and residing in Costa Rica. Informed consent was requested and stored at the "Unidad de ADN, OIJ, Poder Judicial de Costa Rica".

Genomic DNA was isolated from total blood using standard proteinase K-digestion and Chelex extraction (Singer and Tanguay 1989). Amplification was performed in a GeneAmp 9 600 thermocycler. The presence of PCR product was determined with an aliquot in a 1 % agarose minigel in TBE 0.5 X buffer. DNA hybridization and genotyping was performed using the Amplitype PM+HLA-DQA1 kit according to the manufacturer's recommendations (Anonymous 1995).

Gene frequencies were determined by gene counting and maximum likelihood methods. We tested the goodness of fit to the Hardy-Weinberg equilibrium on genotypic data. Some statistical parameters of forensic interest (*h*, heterozygosity; PD, power of discrimination; and CE, *a priori* chance of exclusion) were also calculated. Data from Nicaragua and Costa Rica were analyzed for genetic structure by the exact test of population differentiation. Data analyses were per-

formed with the Arlequin program (Schneider *et al.* 1997). Summary data were banked in the Spanish, Portuguese and Latin American Nuclear DNA Database (Alonso and Albarrán 2000, Anonymous 2001b).

The available information on the allele frequencies of the studied *loci* was used to estimate the genetic distances between several relevant populations. Seven populations of Hispanic origin in the Americas were included in the analysis: Buenos Aires from Argentina (Padula *et al.* 1999), Santiago from Chile (Jorquera and Budowle 1998), Bogota from Colombia (Castillo *et al.* 1996, Terreros-Ibanez *et al.* 1999), Costa Rica (Morales-Cordero *et al.* 2001), Nicaragua (this paper), and two Hispanic groups from the USA (Budowle *et al.* 1995). Two related Spanish populations were considered: Andalusia (Lorente *et al.* 1997, Anonymous 1998a), and Madrid (Herrera *et al.* 1996, Anonymous 1998b). Other populations such as Japan (Anonymous 1995), Korea (Woo and Budowle 1995), and Afro-Americans and Caucasians from the USA (Budowle *et al.* 1995) were also included as reference (Appendix A). An F_{ST} -based distance (Reynolds *et al.* 1983) was computed between every pair of populations. Genetic trees were generated from the distance matrix by means of the neighbor-joining algorithm (Saitou and Nei 1987). A few branches that obtained negative numbers were set to zero. A tree was drawn using the Tree View program (Page 1998). A bootstrap analysis was produced on 1 000 resamples, drawn at random with replacement from the allele set. The standard deviation of these bootstrapped distances was used to estimate both the standard error of the genetic distances and tree robustness. Every occurrence of a particular cluster in the tree was recorded and given as a percentage of the 1 000 bootstrap trees. Percentages above 50 % were regarded as indications of the statistical robustness of a cluster. This analysis was done using the PHYLIP 3.5 package (Felsenstein 1989).

TABLE 1
Allele frequencies of the LDLR, GYPA, D7S8, HBGG, GC, and HLA-DQA1 loci in the Nicaraguan population

Allele	LDLR	GYPA	D7S8	HBGG	GC	HLA-DQA1
A	0.5400	0.6100	0.6750	0.4750	0.1900	
B	0.4600	0.3900	0.3250	0.5000	0.3800	
C				0.0250	0.4300	
1.1						0.1050
1.2						0.1750
1.3						0.0150
2						0.0550
3						0.3550
4						0.2950
Hex	0.4993	0.4782	0.4410	0.5264	0.6378	0.7395
Hob	0.480	0.420	0.470	0.600	0.590	0.700
PD	0.6234	0.6120	0.5887	0.6598	0.7893	0.8965
CE	0.1867	0.1813	0.1712	0.2183	0.3423	0.5235
P*	0.7356	0.2409	0.4764	0.2086	0.7609	0.9161
P**	0.8376	0.2908	0.6486	0.1746	0.7038	0.8522

Abbreviations: Hex, expected heterozygosity; Hob, observed heterozygosity; PD, power of discrimination; CE, *a priori* chance of exclusion; P*, Hardy-Weinberg equilibrium (chi-square test); P**, Hardy-Weinberg equilibrium (exact test based on 100 000 shufflings).

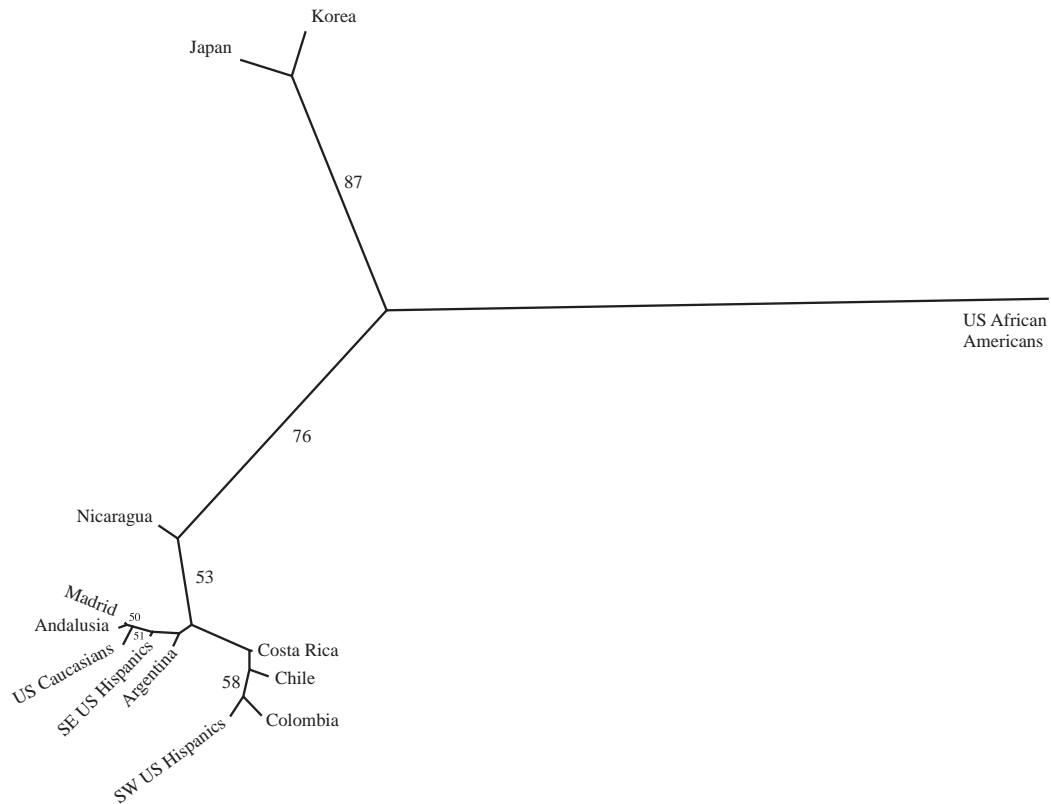


Fig. 1. Neighbor-joining tree based on Reynolds's genetic distances. Numbers represent the percentage of a certain node in 1 000 bootstrapped trees.

TABLE 2
Genetic distance matrix for the populations compared in Fig. 1

	ARG	CSR	CHI	COL	NIC	SEH	SWH	USA	USC	AND	MAD	JAP	KOR
ARG	0.0095												
CSR	0.0066												
CHI	0.0043	0.0083											
COL	0.0139	0.0099	0.0183										
NIC	0.0196	0.0066	0.0164	0.0131									
SEH	0.0068	0.0108	0.0066	0.0208	0.0223								
SWH	0.0131	0.0041	0.0066	0.0050	0.0158	0.0196							
USA	0.1085	0.1093	0.1321	0.1357	0.0920	0.0954	0.1299						
USC	0.0092	0.0143	0.0141	0.0206	0.0219	0.0047	0.0234	0.1019					
AND	0.0074	0.0163	0.0163	0.0236	0.0292	0.0031	0.0243	0.1061	0.0033				
MAD	0.0048	0.0147	0.0135	0.0219	0.0275	0.0036	0.0208	0.1089	0.0030	0.0014			
JAP	0.0667	0.0723	0.0989	0.0959	0.0661	0.0554	0.0900	0.0966	0.0753	0.0747	0.0755		
KOR	0.0616	0.0736	0.0995	0.0977	0.0729	0.0523	0.0895	0.0943	0.0768	0.0711	0.0705	0.0101	

Populations: ARG, Buenos Aires, Argentina; CSR, Costa Rica; CHI, Santiago, Chile; COL, Bogota, Colombia; NIC, Nicaragua; SEH, Southeastern Hispanics; SWH, Southwestern Hispanics; USA, African Americans; USC, Caucasian Americans; AND, Andalusia; MAD, Madrid; JAP, Japan; KOR, Korea.

RESULTS

The calculated statistical parameters of forensic interest are shown in Table 1. The studied systems did not reveal any significant deviation to the Hardy-Weinberg equilibrium. The combined forensic probability of discrimination with those DNA markers is 0.9996, and the probability of excluding a non-father if the mother is known is 0.865.

Significant differences were found between the Nicaraguan and Costa Rican populations (Morales-Cordero *et al.* 2001) at the HLA-DQA1 and GYPA markers ($p < 0.05$). However, there were no statistical differences between both populations at the LDLR, HBGG, D7S8, and GC *loci*.

The genetic distances between the population groups of Hispanic origin from the Americas, the two related groups from Spain, and the other populations used for reference are given in Table 2. All distance values are at least twice their standard errors and thus different from zero. The unrooted tree produced using the neighbor-joining algorithm is shown in Fig. 1. As expected, Afro-Americans split alone like an outgroup, and Japan and Korea formed a cluster together. All Hispanic-derived populations roughly clustered jointly with the Caucasian populations from Spain and the USA. In this context, the Nicaraguan population was closer to the rest of the admixed populations such as those from Chile, Colombia, Costa Rica, and the USA Hispanics than to the European, Asiatic, or other American populations.

DISCUSSION

The analyzed *loci* set was validated as useful for paternity testing and individual identification in the Nicaraguan population residing in Costa Rica, but a study of highly informative *loci*, like the commercially available short tandem repeats (STRs) is recommended, since the *a priori* probabilities found were high but could not be sufficient in

complex cases such as those involving related individuals.

On the other hand, we chose a neighbor-joining reconstruction to analyze the genetic relationships between the Nicaraguan people and their surrounding populations because it leads to unrooted trees, preventing the direct interpretation of the tree as a series of successive fissions from a known starting point. Certainly, this is not the case in the Hispanic-derived populations of the Americas, which were mainly established by admixture processes (Sans 2000). It is remarkable that the unrooted tree obtained through neighbor-joining (Fig. 1) does not reflect the geographic location of the populations. This apparent anomaly is clearly observed between the populations of Nicaragua and Costa Rica, which, in spite of their vicinity and close relationship throughout history, do not cluster together. In fact, the Costa Rican nation clustered in the same branch with the populations from Chile, Colombia, and southwestern Hispanics from the USA. It is interesting that all these populations are known to share similar degrees of admixture (Long *et al.* 1991, Cerda-Flores *et al.* 1994, Sandoval *et al.* 1993, Morera and Barrantes 1995, Merriwether *et al.* 1997, Palomino *et al.* 1997), a factor that shall be influencing the tree topology. Hence, based on the present data, we hypothesize that the Nicaraguan population has a unique proportion of admixture from the ethnic Amerindian, West African, and Spanish ancestral populations, different from the populations studied this far.

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RESUMEN

Los nicaragüenses se han convertido en el grupo minoritario más numeroso y creciente en Costa Rica y en la actualidad representan alrededor del 6 % de la población total del país. Analizamos las frecuencias alélicas y genotípicas de seis marcadores genéticos (LDLR, GYPA, HBG, D7S8, GC y HLA-DQA1) basados en la PCR en 100 nicaragüenses no emparentados, residentes en Costa Rica. Todos los *loci* estudiados cumplieron con el equilibrio de Hardy-Weinberg. También se calcularon algunos parámetros estadísticos de interés forense (*h*, PD y EC). Se encontró que las frecuencias alélicas de los marcadores HLA-DQA1 y GYPA presentan diferencias significativas entre las poblaciones de Nicaragua y Costa Rica. Sin embargo, el análisis de distancias genéticas mostró que la población de Nicaragua es cercana a otras de origen hispano mestizo como las poblaciones de Argentina, Chile, Colombia, Costa Rica y los hispanos de Estados Unidos. Este conjunto de *loci* fue validado como útil para la realización de pruebas de paternidad y para la identificación de individuos en la población nicaragüense residente en Costa Rica.

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APPENDIX A
Allele frequency distribution for PCR-based loci in several populations used in computing genetic distances

Allele N	ARG (109)	CSR (1204)	CHI (130)	COL (151)	NIC (100)	SHE (94)	SWH (96)	USA (200)	USC (200)	AND (235)	MAD (316)	JAP (89)	KOR (116)
LDLR A	0.450	0.531	0.547	0.609	0.540	0.415	0.563	0.235	0.448	0.455	0.433	0.202	0.164
LDLR B	0.550	0.469	0.453	0.391	0.460	0.585	0.438	0.765	0.552	0.545	0.567	0.789	0.836
GYP A	0.610	0.616	0.574	0.672	0.610	0.532	0.656	0.527	0.530	0.504	0.536	0.517	0.534
GYP B	0.390	0.384	0.426	0.328	0.390	0.468	0.344	0.473	0.470	0.496	0.464	0.483	0.466
HBGG A	0.459	0.372	0.434	0.417	0.475	0.426	0.344	0.439	0.537	0.487	0.487	0.331	0.289
HBGG B	0.532	0.571	0.531	0.550	0.500	0.548	0.609	0.228	0.450	0.498	0.505	0.669	0.711
HBGG C	0.009	0.058	0.035	0.033	0.025	0.027	0.047	0.333	0.013	0.015	0.008	0.000	0.000
D7S8 A	0.564	0.638	0.671	0.623	0.675	0.585	0.682	0.655	0.610	0.557	0.571	0.612	0.513
D7S8 B	0.436	0.363	0.329	0.378	0.325	0.415	0.318	0.345	0.390	0.443	0.429	0.388	0.487
GC A	0.321	0.201	0.229	0.235	0.190	0.277	0.271	0.090	0.275	0.311	0.320	0.287	0.284
GC B	0.202	0.258	0.166	0.212	0.380	0.223	0.208	0.720	0.178	0.177	0.153	0.471	0.474
GC C	0.477	0.541	0.605	0.553	0.430	0.500	0.521	0.190	0.547	0.513	0.527	0.242	0.241
DQA1 1.1	0.152	0.144	0.127	0.166	0.105	0.181	0.141	0.125	0.158	0.181	0.131	0.084	0.155
DQA1 1.2	0.116	0.145	0.085	0.123	0.175	0.154	0.135	0.329	0.190	0.146	0.163	0.118	0.121
DQA1 1.3	0.090	0.081	0.030	0.050	0.015	0.080	0.031	0.058	0.073	0.084	0.076	0.236	0.116
DQA1 2	0.076	0.085	0.123	0.083	0.055	0.160	0.094	0.130	0.145	0.178	0.161	0.006	0.039
DQA1 3	0.202	0.246	0.235	0.282	0.355	0.191	0.229	0.090	0.192	0.136	0.156	0.444	0.362
DQA1 4	0.364	0.299	0.400	0.298	0.295	0.234	0.370	0.268	0.242	0.276	0.314	0.112	0.207

N refers to the number of individuals in each database; Populations: ARG, Buenos Aires, Argentina; CSR, Costa Rica; CHI, Santiago, Chile; COL, Bogota, Colombia; NIC, Nicaragua; SEH, Southeastern Hispanics; SWH, Southwestern Hispanics; USA, African Americans; USC, Caucasian Americans; AND, Andalusia; MAD, Madrid; JAP, Japan; KOR, Korea.