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Short population report

4-Locus high-resolution HLA allele and haplotype frequencies in admixed population from Nicaragua

Esteban Arrieta-Bolaños[a,b,c,*], Juan José Madrigal-Sánchez[d], Jeremy E. Stein[b], María José Moreira-Espinoza[e], Edel Paredes-Carias[e], Yondra Vanegas-Padilla[e], Lizbeth Salazar-Sánchez[d], J. Alejandro Madrigal[b,f], Steven G.E. Marsh[b,f], Bronwen E. Shaw[b,g]

[a] Institute for Experimental Cellular Therapy, University Hospital, Essen, Germany
[b] Anthony Nolan Research Institute, Royal Free Hospital, London, UK
[c] Centro de Investigaciones en Hematología y Trastornos Afines (CIHATA), Universidad de Costa Rica, San José, Costa Rica
[d] Escuela de Medicina, Universidad de Costa Rica, San José, Costa Rica
[e] Departamento de Ciencias Morfológicas, Universidad Nacional Autónoma de Nicaragua, León, Nicaragua
[f] UCL Cancer Institute, Royal Free Campus, London, UK
[g] Center for International Blood and Marrow Transplant Research, Department of Medicine, Medical College of Wisconsin, Milwaukee, USA

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ABSTRACT

A total of 155 Nicaraguan Mestizos from across the country were genotyped at high-resolution for the human leukocyte antigen loci HLA-A, -B, -C, and -DRB1 using sequence-based typing methods. The respective allele and extended haplotype frequencies, as well as Hardy-Weinberg proportions were calculated. The most frequent extended haplotype identified was A*24:02:01-B*40:02:01-C*03:05-DRB1*04:07:01G, with an estimated frequency of 2.26%. No deviation from Hardy-Weinberg Equilibrium was detected at any of the loci studied. The HLA genotypic data of the population sample reported here are available publicly in the Allele Frequencies Net Database under the population name “Nicaragua Mestizo” and the identifier AFN3610.

Nicaragua is a Central American country with an area of 131,000 km^2 and a mainly Spanish-speaking population of approximately 6 million inhabitants. Most of the Nicaraguan population resides along the Pacific coast, central, and northern regions, whereas the Atlantic region is more scarcely populated. Important emigrant Nicaraguan populations also exist, especially in Costa Rica and the USA, with which the total Nicaraguan and Nicaraguan-descendant population reaches more than 7 million. Like other Central and Latin American countries, the majority of the population is admixed (Mestizo). Nicaraguan Mestizos (NICA) arose after the 16th century as a mixture of mainly male Spanish colonizers [1], various indigenous peoples, and African slaves introduced to the country after the Spanish conquest. Previous analyses [2] have suggested a differential admixture process took place in Nicaraguans when compared to populations from other Latin American countries such as Colombia, Chile, or Costa Rica, or to Hispanics from the south-western USA.

A total of 155 peripheral blood or saliva samples from unrelated healthy volunteer donors were included in this study. All donors were Nicaraguan Mestizos, excluding individuals from minorities (e.g. Afro-descendants, Amerindians) based on self-identification. Mestizo individuals originated in 14 of the 17 Nicaraguan Departments and were recruited by the National Autonomous University of León, Nicaragua. Samples were collected as part of the DNA biobank at the University of Costa Rica’s Centre for Research in Hematology and Related Disorders (Centro de Investigaciones en Hematología y Trastornos Afines, CIHATA), which continuously collects samples for the characterization of genetic variation across the country and its association with medically-relevant traits. DNA was extracted from blood or saliva by routine methods. All participants gave informed written consent as per CIHATA’s DNA biobank standard procedures. Sample collection under CIHATA’s biobank and this study were approved by the local ethics committees at the University of Costa Rica and the National Autonomous University of León.

High-resolution HLA typing was performed at Anthony Nolan by in house sequence-based typing methods, with generic amplification of exons 2, 3 and 4 for HLA-A, HLA-B, and HLA-C. For HLA class II, exon 2

Abbreviations: AFND, Allele Frequencies Net database; EM, expectation-maximization; HWE, Hardy-Weinberg equilibrium; NICA, Nicaraguan Mestizos

* Corresponding author at: Institute for Experimental Cellular Therapy, Universitätsklinikum Essen, Virchowstraße 171, Essen 45147, Germany.
E-mail address: esteban.arrieta-bolanos@uk-essen.de (E. Arrieta-Bolaños).

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of the HLA-DRB1 gene was amplified using in house allele group-specific primer pairs. Amplicons were purified and each exon was sequenced on an ABI 3730xL DNA Analyzer with specific forward and reverse primers using the Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequence analysis was done with Assign-SBT software (version 3.6+, Conexio Genomics, Freemantle, Australia) using IPD-IMGT/HLA database release 3.9.0 [3]. Ambiguities were further solved using allele-group- (HLA class I) or codon-86-specific (HLA class II) primer combinations and sequencing of the allelic products. For HLA class I, alleles with identical sequences at codons 2, 3, and 4 could not be distinguished, and were assigned the third-field name of the allele with the lowest numerically ordered name (which is usually the more common one). Hence, HLA class I allele frequencies given may in many cases represent the frequency of a group of alleles sharing sequences at these exons. Allele groups (G) were assigned to HLA class II alleles according to IPD-IMGT/HLA database specifications. All homozygous samples were confirmed by at least two determinations and using different techniques. Allele frequencies and compliance with Hardy-Weinberg equilibrium (HWE) were computed using the online tools available from the HLA-net platform (http://hla-net.eu/tools/) [4]. Haplotype frequencies were estimated by an in house implementation of the expectation-maximization (EM) algorithm [5].

All samples were successfully typed for HLA-A, HLA-B, HLA-C, whereas 154 were successfully typed for HLA-DRB1. A total of 33, 61, 30, and 40 alleles were identified for HLA-A, -B, -C, and -DRB1, respectively. Compliance with HWE was confirmed for all loci ($p > 0.5$). The five most common alleles per locus were A*02:01:01 (19.3%), A*24:02:01 (13.9%), A*31:01:02 (8.4%), A*68:01:02 (5.8%), A*03:01:01 (5.5%); B*40:02:01 (10.6%), B*35:01:01 (9.0%), B*07:02:01 (6.1%), B*51:01:01 (4.5%), B*14:02:01, B*35:17:01 and B*44:03:01 (all three 3.9%); C*04:01:01 (16.1%), C*07:02:01 (14.2%), C*03:05 (9.0%), C*01:02:01 (8.7%), C*07:01:01 (6.4%); DRB1*04:07:01G (16.9%), DRB1*07:01:01G (6.8%), DRB1*03:01:01G (5.5%), DRB1*04:03:01 (5.2%), and DRB1*13:02:01 (4.9%). The complete lists of alleles for each locus are given in Supplementary Table 1. The HLA alleles of NICA show clear evidence of the presence of tri-ethnic admixture of its parental populations, with alleles from essentially putative European (e.g. A*25:01:01, B*37:01:01, B*35:08:01), Amerindian (e.g. A*02:22, B*35:43:01, C*03:05), and Sub-Saharan African (e.g. A*02:02, B*15:03:01, DRB1*11:01:02) origin found in the NICA sample.

Based on the results for HLA typing, 4-locus haplotype frequency estimations based on the expectation-maximization algorithm were generated. The number of the extended haplotypes with an estimated frequency > 1/2N was 249. Four extended haplotypes have estimated frequencies of > 1%, with the most common being A*24:02:01-B*40:02:01-C*03:05-DRB1*04:07:01G (2.26%), A*68:01:02-B*40:02:01-C*03:05-DRB1*04:03:01 (1.59%), A*68:03:01-B*35:43:01-C*01:02:01-DRB1*04:07:01G (1.59%), A*02:01:01-B*07:02:01-C*07:02:01-DRB1*15:01:01G (1.08%), and A*31:01:02-B*40:02:01-C*03:05-DRB1*04:07:01G (0.97%). A complete list of estimated extended haplotypes is given in Supplementary Table 2. The HLA profile of the NICA is comparable to recent second-field HLA frequency data obtained in blood donors from the general population in the nation's capital city of Managua [6], and shows a similar proportion of extended haplotypes of likely European (43%) and Amerindian origin (41%) [7], following admixture proportion estimations obtained with other genetic markers [8–10]. All genotype, as well as haplotype and allele frequency data are available in the Allele Frequencies Net database (AFND) [11] under the population name "Nicaragua Mestizo" and the identifier (AFN3610). Haplotype and allele data are available in "G" notation in the Supplementary information accompanying this publication. However, the notation "G" is omitted in AFND due to format restrictions, and second-field level resolution is shown for the relevant alleles instead.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.05.004.

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