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Sequence-based HLA-A, B, C, DP, DQ, and DR typing of 714 adults from Colombo, Sri Lanka

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Abstract

DNA sequence-based typing at the HLA-A, -B, -C, -DPB1, -DQA1, -DQB1, and -DRB1 loci was performed on 714 healthy adult blood bank donors from Colombo, Sri Lanka, to characterize allele frequencies in support of studies on T cell immunity against pathogens, including Dengue virus. Deviations from Hardy Weinberg proportions were not detected at any locus. Several alleles were found in >30% of individuals, including the class II alleles DPB1*04:01, DPB1*02:01, DQB1*06:01 and DRB1*07:01, and the class I alleles A*33:03 and A*24:02. Genotype data will be available in the Allele Frequencies Net Database.

Keywords

HLA alleles; HLA typing; Colombo; Sri Lanka; Sinhalese; Tamil; Moors

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Appendix A. Supplementary Data

Supplementary Table I: Primers used for typing

Supplementary Table II: Allele and phenotype frequencies in Sri Lanka, Colombo (AFND population no. 3423)

Supplementary Table III: Haplotype frequencies in Sri Lanka, Colombo (AFND population no. 3423)

Colombo, located on the western coast of Sri Lanka, is that nation's financial center. With a population of over 750 thousand (2011 census), it is the island's largest city, and the focus of a metropolitan area with a population of about 5.5 million. Because of its natural harbor, Colombo has been well-known for over 2000 years.

The Colombo district has a multi-ethnic population, of which the largest fraction (about 41%; Department of Census and Statistics) is represented by Sinhalese, an Indo-Aryan group native to Sri Lanka, and which also constitutes about 75% of the island's total population (2011 census). Sri Lankan Tamils, native to the island since at least the 2nd century BCE, are distinct from, but closely related to, Sinhalese, and represent another 29%. Sri Lankan Moors, considered a Tamil subset by some, and descendants of 8th–15th century Arab traders by others, comprise about 24% of the Colombo population. Sinhala (Ethnologue three-letter language code, sin) and Tamil (tam) are the two official languages, with Sinhala being the more widely spoken. English (eng) is also spoken by a large fraction of the population.

Anonymous blood donations from 714 adults were obtained from healthy adult blood donors by the National Blood Transfusion Service (NBTS), Ministry of Health, Colombo, Sri Lanka, in an anonymous fashion as previously described [1]. Donors were of both sexes, from the general population, and between 18 and 60 years old. NBTS requires all donors to be healthy, weigh > 50 kilos, with hemoglobin levels >12 g/dL, and have valid identification (pregnant women are excluded). According to NBTS2014 annual statistics report, about 1.8 per cent of the Sri Lankan population voluntary donated blood in 2014, out of which 77% were male and 23% were female. Samples were collected from all 26 districts and were selected at random for the study.

Because all samples were discarded buffy coats from routine anonymous blood donations, they are exempt from human subject review and need for written consent. According to local standards, however, the institutional review boards of both the La Jolla Institute for Allergy and Immunology and the Medical Faculty of the University of Colombo (which served as a National Institutes of Health–approved institutional review board for Genetech), approved all protocols.

Peripheral blood mononuclear cells (PBMCs) and serum were purified by density gradient centrifugation (Ficoll-Paque Premium, GE Healthcare Biosciences, Kowloon, Hong Kong). Cells were then resuspended in SynthaFreeze (Gibco Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA), and cryopreserved in liquid nitrogen [1 2].

HLA-A, -B, -C, -DPB1, -DQA1, -DQB1, and -DRB1 genotyping using locus-specific PCR amplification on genomic DNA was performed for all donors by an American Society for Histocompatibility and Immunogenetics (ASHI)-accredited laboratory at The Institute for Immunology and Infectious Diseases (IIID) at Murdoch University, Western Australia. The assay was adapted from a previously published protocol for Barcoded-PCR method [3] with modifications to the primer sequences (Supplemental Table I). Briefly, genomic DNA was isolated from donor PBMCs using QIAmp DNA isolation kits (Qiagen, Valencia, CA, USA). Eleven amplifications per sample were set up with primers for a given patient sample tailed

with a specific barcode tag sequence. Amplified products were quantitated, normalized and pooled by subject and up to 48 subjects were pooled. The pooled and normalized PCR reactions were purified using 1.8X the PCR reaction volume of Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). Samples were prepared for sequencing on either FLX 454 or Illumina MiSeq using the manufacturer's standard library preparation protocol. These libraries were quantified using Kapa universal QPCR library quantification kits (Kapa Biosystems, Wilmington, MA, USA). Sequencing was performed using either a Roche 454 FLX + sequencer with titanium chemistry (Roche 454 Life Sciences, Branford, CT, USA) or an Illumina MiSeq using a 2 × 300 paired-end chemistry kit (Illumina, San Diego, CA, USA). Reads were quality-filtered, separated by MID tags and alleles called using an in-house accredited HLA allele caller software pipeline that minimizes the influence of sequencing errors. Alleles were called using the IMGT HLA allele database v. 3.21.0 (www.ebi.ac.uk/ipd/imgt/hla) as the reference library[4]. Ambiguities were resolved during the original typing using a proprietary allele-calling algorithm and analysis pipeline and the latest IMGT HLA allele database. All 714 donors were typed at all 7 loci.

Allele frequencies for each locus were determined by direct counting (Supplemental Table II). The most frequent specificities (>0.15) were the class II alleles DPB1*04:01, DPB1*02:01, DQB1*06:01 (and alpha chain alleles DQA1*01:01, DQA1*01:03 and DQA1*02:01) and DRB1*07:01, and the class I alleles A*33:03 and A*24:02. The relatively rare allele A*02:11 was present with a frequency of 0.066, making it the most frequent A*02 split, confirming a previous Colombo Sinhalese study[5] suggesting a North Indian origin for this group.

Haplotype frequencies (i.e. A~B~C~DPB1~DQA1~DQB1~DRB1, Supplemental Table III) were estimated using an iterative Expectation-Maximization (EM) algorithm implemented in BIGDAWG (version 1.16)[6]. From a total of 950 unique haplotypes identified, A*33:03~B*44:03~C*07:01~DPB1*02:01~DQA1*02:01~DQB1*02:02~DRB1*07:01 (frequency 1.4%) was the most common. Four other haplotypes were also present with frequencies of 1.3%.

Adherence to Hardy-Weinberg equilibrium proportions did not reveal deviations ($p < 0.05$) for any locus. The number of unique HLA-DPB1, -DQA1, -DQB1 and -DRB1 alleles was 30, 8, 21 and 43 respectively, and the number of unique HLA-A, -B and -C alleles was 26, 55, and 32, respectively (see Supplemental Table II). In total, 215 unique alleles were identified in the Colombo cohort.

The frequency and genotype data is available in the Allele Frequencies Net Database under population Sri Lanka Colombo (AFND population identifier 3423)[7].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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