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Sequence-based HLA-A, B, C, DP, DQ, and DR typing of 100 Luo infants from the Boro area of Nyanza Province, Kenya

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Abstract

One hundred healthy infants enrolled as controls in a tuberculosis vaccine study in Nyanza Province, Kenya provided anonymized samples for DNA sequence-based typing at the HLA-A, -B, -C, -DPB1, -DQA1, -DQB1, -DRB1, and -DRB3/4/5 loci. The purpose of the study was to characterize allele frequencies in the local population, to support studies of T cell immunity against pathogens, including *Mycobacterium tuberculosis*. There are no detectable deviations from Hardy Weinberg proportions for the HLA-B, -C, -DRB1, -DPB1, -DQA1 and -DQB1 loci. A minor deviation was detected at the HLA-A locus due to an excess of HLA-A*02:02, 29:02, 30:02, and 68:02 homozygotes. The genotype data are available in the Allele Frequencies Net Database under identifier 3393.

Keywords

HLA alleles; Kenya; Luo

Boro is a community in Nyanza Province in western Kenya, immediately east of Lake Victoria. It is populated by one tribal group, the Luo, who have historical roots in the Lake Victoria region. The Kenyan Luo Tribe is a subgroup of the larger Luo community that

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spans across Uganda, Tanzania, Sudan, Congo and Ethiopia. The Luo tribe is the fourth largest community in Kenya and makes up close to 15% of the entire population, estimated to be 4 million in 2009. The Luo population is descended from Sudan, traveling along the River Nile around 500 years ago. Through intermarriages and wars the Luo are part of the genetic admixture that includes all modern East African ethnic groups. Currently the Luo community has 26 sub groups. Dholuo (Ethnologue three-letter language code, *luo*) is the main spoken language, but English (*eng*) and Swahili (*swh*) are also spoken.

A parent or guardian of each infant participant provided informed consent for enrollment into an Aeras Tuberculosis (TB) Vaccine Study and for blood samples to be stored and used for additional research. The study and informed consent documents were reviewed and approved by the Kenya Medical Research Institute Ethics Review Committee.

Samples used for this analysis were collected as controls from a TB vaccine study and anonymized prior to shipment from Aeras. All samples selected for this analysis were from participants whose parent or guardian self-reported as being from the Luo ethnic group. Blood was collected from 100 healthy infants enrolled in the study and peripheral blood mononuclear cells (PMBC) were processed and frozen within 8 h of collection. Siblings were not enrolled in the study; other relationships between participants are unknown.

HLA-A, -B, -C, -DPB1, -DQA1, -DQB1, -DRB1, and -DRB3/4/5 genotyping using locus-specific PCR amplification on genomic DNA was performed by an American Society for Histocompatibility and Immunogenetics (ASHI)-accredited laboratory at The Institute for Immunology and Infectious Diseases at Murdoch University Western Australia. The assay was adapted from a previously published protocol for Barcoded-PCR method [1] with modifications to the primer sequences (Supplemental Table I). Briefly, 11 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 1.8× the PCR reaction volume of Agencourt AMPure XP beads (Beckman Coulter Inc. 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, Inc. 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, Inc, San Diego, CA, USA). Reads were quality-filtered, separated by MID tags and alleles were 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 (http://www.ebi.ac.uk/ipd/imgt/hla/) as the allele reference library [2].

Allele frequencies for each locus were determined by direct counting (Supplemental Table II). Haplotype frequencies (e.g. A ~ B ~ C ~DPB1 ~ DQA1 ~ DQB1 ~ DRB1, Supplemental TAble III) were estimated using iterative Expectation-Maximization (EM) algorithm, implemented in BIGDAWG [3]. These frequencies are available (along with the genotype data) in the Allele Frequencies Net Database under identifier 3393.

Adherence to Hardy-Weinberg equilibrium proportions (HWEP) were assessed using PyPop [4], and revealed no detectable deviations from HWEP for the HLA-B, -C, -DRB1, -DPB1, -DQA1 and -DQB1 loci. A minor (p-value = 0.0329) deviation was detected at the HLA-A locus due to an excess of HLA-A*02:02, 29:02, 30:02, and 68:02 homozygotes.

These genotype data are available in the Allele Frequencies Net Database under the population name, “Kenya Luo” and the identifier: 3393 [5].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2017.03.007.

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