Genetic Mechanisms Involved in the Generation of HLA Alleles in Brazilians: Description and Comparison of HLA Alleles

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ABSTRACT

Background. The HLA genes show high levels of diversity as indicated by the number of HLA alleles. There are almost 11,000 classical HLA-A, -B, -DRB1 alleles in populations around the world, making the search for compatible donors difficult. HLA diversity is generated by different genetic mechanisms, such as point mutations, which result in single nucleotide polymorphisms, insertion and deletion, and recombination. The aim of this study was to describe genetic mechanisms involved in the generation of HLA alleles in Brazilians.

Methods. Twenty-six alleles indentified in the Brazilian bone marrow donors were include in the study. Data regarding new HLA alleles by sequence-based typing were also used to elucidate what genetics mechanism was involved in the HLA variability. The new alleles were officially named by the World Health Organization Nomenclature Committee.

Results. The new alleles described were HLA-DRB1*11:04:14, HLA-A*33:117, and HLA-B*41:48. The DRB1*11:04:14 allele was generated by synonymous point mutation at codon 48. The A*33:117 allele was generated by nonsynonymous nucleotide mutation leading to amino acid substitution at codon 74. The B*41:48 allele was generated by an intralocus gene conversion between the HLA alleles from groups HLA-B*13, B*35, B*53, or B*58 and an allele from the HLA-B*41 group.

Conclusions. Different genetic mechanisms introduce new mutant HLA alleles into the human population requiring attentive and rigorous specialists and the use of different methodologies to identify these mutations in HLA typing routine.

THE HIGH level of gene variability among HLAs makes each individual genetically unique [1]. The HLA genes show high levels of diversity as indicated by the large number of HLA alleles and the high heterozygosity observed in the populations (more than 85%) [2]. The notably high level of polymorphism and the distribution pattern of HLA alleles and haplotypes in human populations worldwide also make the search for compatible organ and hematopoietic stem cell donors difficult [1]. According to the Immuno Polymorphism Database–International Immunogenetics Project Database, almost 11,000 classical HLA-A, -B, -DRB1 alleles have been identified in populations around the world [3], officially recognized and named by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System.

HLA diversity is generated by different genetic mechanisms, such as point mutations as single nucleotide polymorphism (SNP), insertion and deletion, and recombination events (gene conversion and crossing over) [4,5]. The SNPs can be synonymous or nonsynonymous, and insertion and deletion of single nucleotides can cause a frameshift in the messenger RNA, resulting in a premature

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stop codon. Gene conversion and crossing over are the results of recombination events in which a bidirectional exchange of sequence fragments occurs between 2 homologous chromosomes. In gene conversion, which may be intragenic or intergenic, part of the nucleotide sequence from one allele is replaced by the homologous nucleotide sequence from another allele [5,6]. The accuracy of HLA typing is crucial for the identification of incompatibilities between the recipient and the donor. Numerous methodologies with low ambiguity rates have been developed for a high throughput routine typing [7]. The next-generation sequencing method provides accurate and low-ambiguity HLA typing and allows the detection of rare and new HLA alleles based on the sequences of coding and noncoding regions [7,8].

The decrease in graft survival rates is proportional to the number of HLA mismatches [9,10] because HLA compatibility plays a significant role in reducing graft failure and minimizes the risk of graft rejection by improving the genetic matching between the recipient and the donor [10,11]. New HLA alleles have great clinical importance in the context of transplants because they can increase the probability of complications related to HLA incompatibility. In addition, it is important to know the exact number of HLA mismatches before kidney transplantation to define patient outcome [12] and predict graft-versus-host disease in hematopoietic stem cell transplantation [13,14]. All of these factors emphasize the need for a deeper and more comprehensive analysis of the HLA region during HLA typing. The aim of this study was to describe some genetic mechanisms involved in the generation of HLA alleles in Brazilian individuals.

METHODS

Subjects

We identified 3 novels HLA alleles in 3 Brazilians individuals reported for the first time in this article. Data published by other authors about HLA alleles identified in Brazilians were also used to exemplify the genetic mechanisms involved in the generation of HLA variability. The subjects were male and female unrelated individuals from different ethnic groups who were registered in the Brazilian Bone Marrow Volunteer Donor Registry, the third largest registry in the world with more than 4.3 million donors.

HLA Typing

Genomic DNA was extracted from peripheral blood leukocytes using either the Biopur DNA Extraction Kit according to the manufacturer's instructions (SR Produtos para Laboratório, PR, Brazil) or salting-out technique [15]. The HLA typing was first performed using a LabType polymerase chain reaction-SSOP (sequence-specific oligonucleotide probe, One Lambda, Canoga Park, Calif, United States). These samples yielded unexpected probe patterns; thus, they were typed by sequence-based typing using SeCore HLA sequence-based typing kits (Invitrogen, Foster City, Calif, United States). For all studied sequences, no matches were found with any known HLA allelic combination in the sequence library, suggesting the presence of novel HLA alleles. Monoallelic sequencing strategies were performed using the group-specific Domino Stone and locus-specific Domino Stone single sequencing set (Protrans GmbH, Heidelberg, Germany) and analyzed by SeqPilot (JSI medical systems GmbH, Heidelberg, Germany) according to the manufacturer's instructions.

The names of all new HLA alleles have been officially assigned by the WHO Nomenclature Committee. This follows the agreed policy that names will be assigned to new sequences as they are identified, as governed the conditions stated in the most recent Nomenclature Report [16]. Lists of such new names will be published in the following WHO Nomenclature Report. This study was approved by the ethics committee of the Faculty of Medical Sciences under permit no. 066828/2016.

RESULTS

The point mutation can result in different manners of gene expression. The first was exemplified by synonymous point mutation resulting in no amino acid change in the HLA-DRB1*11:04:14 allele identified in a female voluntary bone marrow donor of mixed ethnicity. The most similar allele was HLA-DRB1*11:04:01; DRB1*11:04:14 differs from this similar allele in one nucleotide at position 231 in exon 2. An exchange from G to A (codon 48, CGG to CGA) (Fig 1A) had occurred; however, both codons encode aspartic acid. The complete HLA type of the donor was HLA-A*01:AGFFU, A*02:AGFGE; HLA-B*18:AEHBF, B*57:AEHGH; HLA-DRB1*04:01, DRB1*11:04:14. The nucleotide sequence of the allele has been submitted to GenBank (accession number KC433572), and its name was officially assigned by the WHO Nomenclature Committee in August 2016. We were unable to study the family members of the donor to determine the HLA haplotype associated with the new HLA allele. Table 1 shows other published HLA alleles that underwent synonymous mutations identified and described in Brazilian individuals [17–29].

The second example of SNP, resulting of the nonsynonymous point mutation leading to an amino acid substitution in the HLA-A*33:117 allele, was identified in a male donor of European descent. The most similar allele was HLA-A*33:03:01. A*33:117 differs from this similar allele at nucleotide 293 in exon 2, where an exchange from A to G (codon 74, GAC to GGC) resulted in an amino acid change from aspartic acid to glycine (Fig 1B and 1C). The complete typing of the patient was HLA-A*01:01, A*33:117; HLA-B*41:MGDZ, B*53:GDDB; HLA-DRB1*07:JXKR, DRB1*08:04. The nucleotide sequence of the allele has been submitted to GenBank (accession number KX611133), and its name was officially assigned by the WHO Nomenclature Committee in August 2016. Table 1 shows other published HLA alleles that underwent nonsynonymous mutation identified and described in Brazilians [17–29].

The third example of SNP was the null HLA-A*02:643N allele identified in a female donor of European descent as reported recently by our group [29]. The sequence-based typing showed that the most similar allele was HLA-A*02:02:01:01; HLA-A*02:643N differs from this similar allele in one nucleotide at position 420 in exon 3, where an