Identification of four novel *RHD* alleles with altered expression of D in Brazilians

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The high similarity as well as the close proximity and opposite orientation of *RHD* and *RHCE* promote molecular rearrangements giving rise to variant alleles. The D variants encoded by these alleles can arise from different mechanisms including nucleotide insertions or deletions, single- or multiple-missense mutations, splice site mutations, or gene conversions.[1] D antigen variants are classified into partial D, weak D, and DEL types and their identification is of clinical relevance. Genetic *RHD* variations differ among ethnic groups and populations. Although there is much of evidence regarding frequency and molecular basis of D variants, the majority of reports comprise Caucasian and African populations.[2] Identification of D variants in admixed populations, like Brazilians, is of interest, since there is an atypical ethnic background. Here, we report four novel *RHD* alleles found in Brazilian blood donors resulting from nucleotide changes.

BRIEF METHODS

The novel *RHD* alleles were identified in a cohort of 360 blood donor samples obtained from different donor centers located in São Paulo State, Brazil. The samples were sent to our laboratory due to weak reactivity or discrepancies among the donations in routine serologic D typing performed with a blend (MS26 + TH28) monoclonal antibody (Bio-Rad) in tube test and gel test. RBCs of four samples with reactivity in indirect antiglobulin test (IAT) only, were further examined by four other anti-D monoclonal reagents: three anti-D IgM, clones RUM-1, MS201 (Lorne, Berkshire, UK), and TH28 (Bio-Rad), and one anti-D blend (RUM-1 + MS26, Lorne). DNA samples were tested by RHD BeadChip (Immucor) and sequencing. DNA sequencing of *RHD* Exons 1 to 10 and flanking intron regions was performed by Sanger method using primers previously reported.[3] For specific detection of the *RHD* gene deletion we used two molecular assays, a polymerase chain reaction (PCR)–restriction fragment length polymorphism (amplification of the downstream and hybrid Rhesus box as well as digestion of the PCR products with the restriction enzyme *PstI*)[4] and a multiplex real-time quantitative PCR.[5] When fresh blood samples were available, cDNA analysis was also performed as described elsewhere.[6] The reagents used in the molecular techniques were obtained from Applied Biosystems.

RESULTS

Table 1 shows information associated with molecular and initial serologic analysis, *RH* haplotypes, zygosity results, and the GenBank accession numbers of the four new *RHD* alleles identified. All samples were obtained from nonrelated Brazilian donors self-identified as Caucasians and were collected in different donors' centers in the same region of Brazil. cDNA analysis, when performed, confirmed the nucleotide changes observed in DNA sequencing.

Alleles *RHD*841C* and *RHD*359A* (identified in Table 1 as 1 and 2) encode amino acid changes predicted to be in the membrane-spanning domains while the allele *RHD*602G,667G,697C,733C,744T,1136T* (ID 3) encodes amino acid changes within the membrane-spanning domain and cytoplasmic loops of the protein and could be included in the African DAU cluster since the c.1136C>T is a SNP shared by *RHD*DAU* variants. DNA and cDNA sequencing showed that *RHD*602G,667G, 744T,957A,1025C,1063A* allele (ID 4) has the same set of SNPs found in the *RHD*weak D Type 4.2.2* (the most common D variant in our population[7]) with the additional change c.1063G>A in Exon 7 and therefore could be included in the African weak D Type 4 Cluster. RHD BeadChip identified the four samples with this variant allele as

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