RHD Positive Haplotype in D Negative Omani Blood Donor

Mujtaba Al Lawati[1]* and Badriya Al Balushi[2]
[1]Department of Medical Lab Sciences, Oman College of Health Sciences, Muscat, Oman
[2]Central Blood Bank, Ministry of Health, Muscat, Oman

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ABSTRACT
The frequency of Rhesus D negative blood group in Omanis is 8.35% but the molecular background of this phenotype is unknown in the Omani population. The Rhesus D negative phenotype has a high molecular diversity. We report a rare case of serological D negative with existence of complete *RHD* gene in a 43-year-old Omani male blood donor. Molecular analysis of *RHD* exons showed duplication across the boundary of intron 3 and exon 4. This is a 37 bp insert in *RHD* exon 4 along with c.609 G>A mutation. We are uncertain if the presence of *RHD Ψ* is homozygous (*RHD Ψ/RHD Ψ*) or hemizygous (*RHD Ψ/del*). Therefore, molecular basis of D zygosity determination would be a good approach to further explore the case.

Among the blood group systems, the Rhesus (Rh) system is the second in clinical importance after the ABO system. The Rh system has two main genes: *RHD* encodes the D antigen and *RHCE* encodes for C/c and E/e antigens both with 10 exons.[1] *RHD* and *RHCE* genes each produce a protein antigen with 417 amino acids long. The most important antigens of the Rh system are D, C.c, E, and e.[2] The immunogenicity of Rh antigens differs, with D antigen being the most immunogenic.[3] To prevent alloimmunization due to anti-D, exposure of D-negative individuals to D-positive red blood cells should be avoided. Therefore, correct D phenotyping of donor's red blood cells is essential to avoid such anti-D alloimmunization.

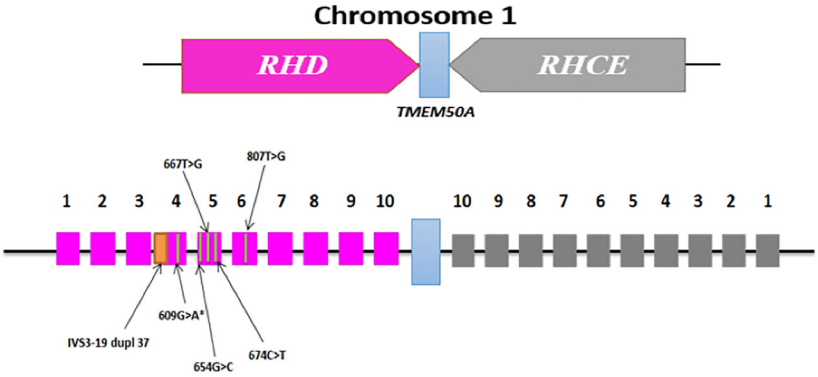
In most laboratories, serology is the method of choice to detect D antigen; however, it has limitations. Studies have shown that D variants such as weak D, Del phenotype, and partial D may be missed by standard serologic methods including the indirect antiglobulin test, and may cause anti-D immunization when transfused to D-negative recipients. Garratty estimated that the blood of at least 120 weak D or Del donors (though serologically type D negative) is transfused to D-negative recipients annually in Southern California.[4] In another study on 46 133 serologically D-negative donors, the *RHD* genotyping showed that 96 samples had *RHD* gene, half of which harbored Del phenotype.[5] Moussa et al,[6] study realized that a partial D sample type DBT was mistyped as D-negative by serological tests. *RHD* gene molecular typing can overcome such limitations of serology.

The D-negative phenotype has a high molecular diversity which explains the discrepancies found between serologic and molecular methods.[7] The frequency of D negative in Omanis is 8.35%,[8] but the molecular background explaining this phenotype is still unknown in this population. With the aim to explore the molecular background of the possibility of serological D negative for any D variants, we describe an unusual case of serological D negative with the presence of an entire *RHD* gene in an Omani blood donor.

CASE REPORT

A 43-year-old B RhD negative Omani male donor passed all eligibility tests and donated blood. Serological Rh phenotyping showed a D-C-c+E-e+ phenotype giving the initial impression of a possible *dce/dce* genotype. For molecular analysis, the presence of *RHD* exons 1 through 7 and *RHD* exons 9 and 10 was observed and found to be positive for all *RHD* exons except *RHD* exon 5. Sequencing of these *RHD* exons ruled out D variants. Sequencing of *RHD* intron 3/exon 4 for RHD pseudogen (*RHD Ψ)* revealed 37 bp insertion with c.609 G>A mutation. This suggests and confirms the presence of the African *RHD* genotype responsible for the serological D-negative phenotype in this donor.

*Corresponding author: ✉mujtaba.mls.ihs@gmail.com



RHD and RHCE genes are separated by a small membrane protein TMEM50A gene. The numbers indicate the exon number on both RHD and RHCE genes. The pink boxes represent RHD exons and the grey boxes represent RHCE exons. The orange band within the pink box of RHD exon 4 represents a 37 bp insert which is a duplication of a sequence spanning the intron 3 (at -19 nucleotide sequence) – exon 4 boundary (IVS-19 dup 37). The green vertical lines represent point mutations associated with RHDΨ gene at RHD exon 4 (609G>A), RHD exon 5 (654G>C, 667T>G and 674C>T), and RHD exon 6 (807T>G). Asterisk (*) indicates a point mutation that does not result in an amino acid change. RHDΨ gene has no effect on RHCE gene.

Figure 1: Schematic diagram on the molecular background of *RHDΨ* gene that gives D-negative phenotype.

The serological D negative was considered a true D negative with a possible *Dce/dce* genotype.

DISCUSSION

The molecular background of D negative has been extensively studied in Caucasians with frequencies between 15% and 17% and Africans with frequencies between 1% and 7%.[9–11] Two molecular backgrounds exist in D-negative Africans: *RHDΨ* and the *RHD-CE-D*^{s} hybrid gene that does not express D antigen but encodes an altered C antigen.[12–14] In most Caucasians, the frequent cause for D negative phenotype is the lack of the entire *RHD* gene.[15]

In the present case, molecular analysis showed the presence of a complete *RHD* gene along with *RHDΨ*. This D negative is predicted to be either hemizygous or homozygous for *RHDΨ* gene. Omani populations are known to have an admixture of African genes,[16] which can present a high variety of *RHD* alleles and explains the existence of *RHDΨ*.

RHDΨ is characterized by inactivation of D gene by insertion of 37 bp at the intron 3/exon 4 boundary of *RHD* gene that introduces a frameshift and translation termination. In addition, a nonsense (Tyr>stop) mutation in exon 6 causes premature termination of translated protein.[12] *RHDΨ* associated nucleotides and amino acids changes related to wild-type *RHD* gene can be viewed in Figure 1. *RHD* gene deletion is a common cause of D negative in Africans, however around 67% are at least heterozygous to *RHDΨ*.[17]

In this case report, a previously described primer pair was used to amplify both wild-type *RHD* and *RHD* with a 37 bp insert specific for *RHDΨ*. The presence of 37 bp insertion was confirmed by Sanger sequencing. A previously described sequence-specific primer for *RHD* exon 5 was designed in a way so that the forward primer 3' specific for wild type c.654 in exon 5 does not amplify mutation G>C (M218I) associated with *RHDΨ*.[18] Therefore, amplification of *RHD* exon 4 and nonamplification of *RHD* exon 5 further confirmed the existence of *RHDΨ*. We were uncertain if the presence of *RHDΨ* is homozygous (*RHDΨ/RHDΨ*) or hemizygous (*RHDΨ./del*). D zygosity testing would have been helpful to unveil the same.

CONCLUSION

This is the first molecularly analyzed case that revealed African *RHDΨ* existence in an Omani blood donor. Our observation drives us to realize the necessity to study the molecular background of D-negative phenotype in Omanis. The molecular basis of D zygosity determination would be a good approach to further explore the case.

Disclosure

The authors declared no conflicts of interest. Informed written consent was taken from the patient.

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