RHD positive haplotype in D negative Omani donor

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Abstract

The frequency of RhD negative in Omanis is 8.35% but the molecular background explaining this phenotype is unknown in this population. The RhD negative phenotype has a high molecular diversity. We describe a case report of serological D negative with existence of complete *RHD* gene in an Omani 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 were uncertain if the presence of *RHDΨ* is homozygous [*RHDΨ./ RHDΨ.*] or hemizygous [*RHDΨ./del*]. Therefore, molecular basis of D zygosity determination would be a good approach to further explore the case.

Introduction

The human Rh blood group system is the most important system clinically after ABO group system. The Rh blood group 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 (RBCs) should be avoided. Therefore, correct D phenotyping of donor´s RBCs is essential to avoid such anti-D alloimmunization.

In most laboratories, serology is the method of choice for the detection of D antigen, however, it has limitations. Studies shown that detection of D variants such as weak D, Del phenotype and partial D may be missed by standard serologic methods including Indirect antiglobulin test (IAT) and may cause anti-D immunization when transfused to D negative recipients. Garratty calculated that at least 120 weak D or Del donors, typed D negative serologically, are transfused to D negative recipients annually in Southern California[4]. In another study by Flegel and colleagues on 46133 serologically D negative donors, the *RHD* genotyping showed that 96 samples had *RHD* gene, half of which harbored Del phenotype[5]. Moussa and colleagues study realized that a partial D sample type DBT was mistyped as D negative by serological tests[6]. The limitations of serology can be overcome by *RHD* gene molecular typing.

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 unknown in this population. In an aim to explore the molecular background of a serological D negative for any D variants possibility, we describe a case report of serological D negative with presence of entire *RHD* gene in an Omani blood donor.

Case report

A 43-year-old B Rh(D) negative Omani male donor passed all eligibility criteria tests and donated blood. Serological Rh phenotyping showed D-C-c+E-e+ phenotype giving initial impression of possible *dce/dce* genotype. For molecular analysis, the presence of *RHD* exons 1 through 7 and *RHD* exons 9 and 10 were 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 *RHDΨ* revealed 37 bp insertion with c.609 G>A mutation. This suggests and confirms the presence of African *RHD* genotype responsible for the serological D negative phenotype in this donor. The serological D negative was considered a true D negative with possible *Dce/dce* genotype.

Discussion

The molecular background of D negative has been extensively studied in Caucasian with frequencies between 15 and 17%[9] and Africans with frequencies between 3 and 7%[10]. Two molecular backgrounds exist in D negative Africans; *RHD* pseudogene (*RHDΨ*)[11] and the *RHD-CE-D^s* hybrid gene that does not express D antigen but encodes an altered C antigen[12-13]. In most Caucasians, the frequent cause is the lack of entire *RHD* gene[14].

We report a case of D negative phenotype with *RHD* positive haplotype in an Omani male donor. Molecular analysis showed the presence of complete *RHD* gene along with *RHDΨ*. This D negative predicted to be either hemizygous or homozygous for *RHDΨ* gene. Omani populations is admixed of African[15] 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 frame shift and translation termination. In addition, a

nonsense (Tyr>stop) mutation in exon 6 that causes premature termination of translated protein[11]. *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 African, however around 67% are at least heterozygous to *RHDΨ*[16].

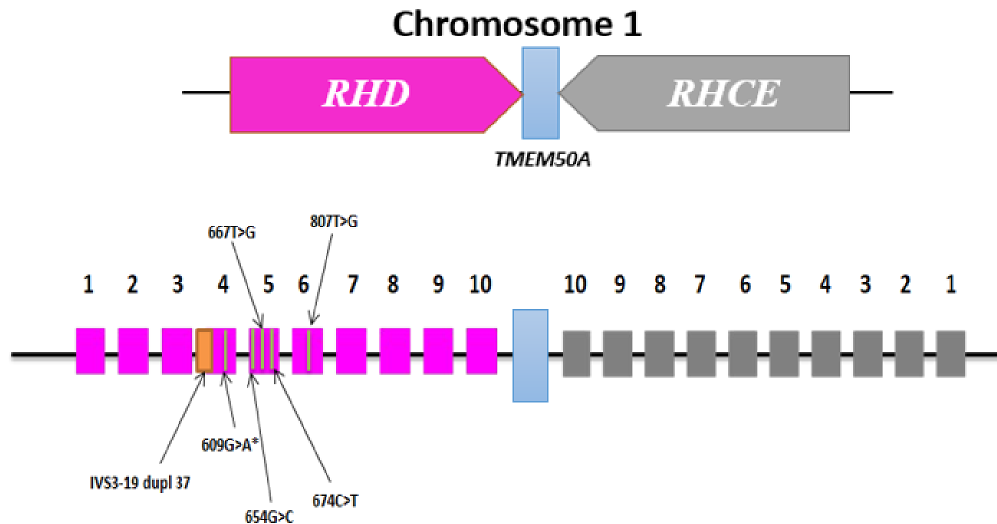

Figure 1: Schematic diagram on molecular background of *RHDΨ* gene that gives D negative phenotype.

RHD and *RHCE* genes separated by small membrane protein *TMEM50A* gene. The numbers indicate exon number on both *RHD* and *RHCE* genes. Pink box represent *RHD* exons and grey box represents *RHCE* exons. Orange box and green lines represent the insertion/mutations associated with existence of *RHDΨ* gene. The orange within pink box of *RHD* exon 4 represents 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). Green 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.

In this case report, a previously described primer pair was used to amplify both wild type *RHD* and *RHD* with 37 bp insert specific for *RHDΨ*. The presence of 37 bp insertion was confirmed by Sanger sequencing. A previously described sequence specific primers (SSP) for

RHD exon 5 designed in a way so the forward primer 3´ specific for wild type c.654 in exon 5 do not amplify mutation G>C (M218I) associated with *RHDΨ*[17]. Therefore, amplification of *RHD* exon 4 and no amplification of *RHD* exon 5 further confirmed existence of *RHDΨ*. We were uncertain if the presence of *RHDΨ* is homozygous [*RHDΨ./ RHDΨ.*] or hemizygous [*RHDΨ./del*], therefore, D zygosity testing would have been very helpful to unveil that.

Conclusion

We report a first molecularly analysed case of African *RHDΨ* existence in Omani donor. Our observation drives us to realize the necessity to study the molecular background of D negative phenotype in Omanis. Molecular basis of D zygosity determination would be a good approach to further explore the case.

References

1. Cherif-Zahar B, Mattei MG, Le Van Kim C, Bailly P, Cartron JP, Colin Y. Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. Hum Genet. 1991;86(4):398-400.
2. Flegel WA. The genetics of the Rhesus blood group system. Blood Transfus. 2007;5(2):50-7.
3. Harmening D. *Modern blood banking & transfusion practices*. (6th ed.). Philadelphia: F. A. Davis Company; 2012.p149-165.
4. Engelfriet C, Reesink HW. Testing for weak D. Vox Sang. 2006;90(2):140-53.
5. Flegel WA, von Zabern I, Wagner FF. Six years' experience performing RHD genotyping to confirm D- red blood cell units in Germany for preventing anti-D immunizations. Transfusion. 2009;49(3):465-71.
6. Moussa H, Tsochandaridis M, Chakroun T, Jridi S, Abdelneji B, Hmida S, et al. Molecular background of D-negative phenotype in the Tunisian population. Transfus Med. 2012;22(3):192-8.
7. Sassi A, Ouchari M, Houissa B, Romdhane H, Abdelkefi S, Chakroun T, et al. RHD genotyping and its implication in transfusion practice. Transfus Apher Sci. 2014;51(3):59-63.

8. Allawati M, Al-Kalbani L, Al-Balushi T. ABO and Rh (D) Phenotypes, Allele Frequencies and Estimated Genotypes in Omanis: A Retrospective Study from Armed Forces Hospital. JMSCR. 2021;9(4):1-7.
9. Westhoff CM. The structure and function of the Rh antigen complex. Semin Hematol. 2007;44(1):42-50.
10. Touinssi M, Chapel-Fernandes S, Granier T, Bokilo A, Bailly P, Chiaroni J. Molecular analysis of inactive and active RHD alleles in native Congolese cohorts. Transfusion. 2009;49(7):1353-60.
11. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, et al. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in africans with the Rh D-negative blood group phenotype. Blood. 2000;95(1):12-8.
12. Faas BH, Beckers EA, Wildoer P, Ligthart PC, Overbeeke MA, Zondervan HA, et al. Molecular background of VS and weak C expression in blacks. Transfusion. 1997;37(1):38-44.
13. Daniels GL, Faas BH, Green CA, Smart E, Maaskant-van Wijk PA, Avent ND, et al. The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. Transfusion. 1998;38(10):951-8.
14. Wagner FF, Flegel WA. RHD gene deletion occurred in the Rhesus box. Blood. 2000;95(12):3662-8.
15. Al-Dughaishi T, Al Harrasi Y, Al-Duhli M, Al-Rubkhi I, Al-Riyami N, Al Riyami A, et al. Red Cell Alloimmunization to Rhesus Antigen Among Pregnant Women Attending a Tertiary Care Hospital in Oman. Oman Med J. 2016;31(1):77-80.
16. Daniels G. Variants of RhD--current testing and clinical consequences. Br J Haematol. 2013;161(4):461-70.
17. Legler TJ, Liu Z, Mavrou A, Finning K, Hromadnikova I, Galbiati S, et al. Workshop report on the extraction of foetal DNA from maternal plasma. Prenat Diagn. 2007;27(9):824-9.