

Pedigree analysis for the rare RHD DVa allele: serological and molecular studies

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ABSTRACT

Until now, worldwide more than 80 different alleles producing weak D phenotypes have been identified. Here we identified rare RHD DVa alleles in Chinese individuals associated with weak expression of D antigen and an RHD phenotype resembling DVI. Multi-monoclonal anti-D antibodies were used to identify the RHD phenotyping for rare RHD DVa. RHD genotyping was used to confirm the presence of RHD exons and identify RHD, RHCE hybrids and exon deficiencies. Sanger sequencing was used to identify nucleotide polymorphisms in RHD exons. Pedigree analysis demonstrated RHD DVa allele alterations of 667 T>G, 676 G>C, 697 G>C, 712 G>A, 733 G>C, 744 C>T and 1227 G>A, which means the proband's alleles were RHD DVa-3 [also called RHD-CE(5)-D] and 1227 G>A. The results also demonstrated RHD DVa and the original RHD Va allele without 1227 G>A. The study suggests that RHD phenotyping is a superior strategy for the molecular analysis of RHD variant in Chinese subjects, and for understanding related polymorphisms and mutations.

Keywords: RHD Va, Del, pedigree analysis

INTRODUCTION

The RH blood group is comprised of the most important and complicated human erythrocytic antigens, with over 50 related antigens, including RHD, RHC, RHc, RHE, and RHe^[1]. The RHD antigen is the most clinically significant antigen in the RH blood group system in clinical transfusion and obstetrics. RHD variants display inconsistent reactions to different monoclonal anti-D antibodies, which include weak D, partial D and D elution, of which molecular analyses have identified more than 150 RHD variant types^[2–5].

In this study, we investigated the molecular genetic basis of the apparently weak D phenotypes observed in a proband and his family members, and described a rare RHD DVa allele.

MATERIALS AND METHODS

Subject

A 46 years old male presented for routine physical examination. Occult blood in his stool was found positive. His health condition was nice without diabetes mellitus, hypertension and other metabolic dis-

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eases, and he was deemed generally healthy. Due to the proband's family history of colorectal carcinoma having stool occult blood, the colonoscopy was arranged.

Serological typing

ABO RH typing was done by using the Wadiana automated system (Grifols Diagnostica. S.A., Barcelona, Spain) and the ABO-RHCDE typing card (Grifols Diagnostica. S.A.), which specifically identifies DVI. Antibody screening was done by using human anti-globin and micro-column testing combined to use Panels 1-3 (Grifols Diagnostica. S.A.). The anti-D confirmation test followed the National Health Commission's specification for Clinical Transfusion Technology. Phenotyping involved the use of monoclonal IgG/IgM mixed anti-D (Anti-D series 4, Gamma-clone, Immucor Inc., USA) comprising of an IgM clone (MS201) and IgG clone (MS26); an anti-D blend (Gamma-clone, Immucor Inc., USA) comprising of an IgM clone (GAMA401) and IgG clone (F8D8); and a Gamma-clone comprising of an anti-D (IgM clone: D175-2, and IgG clone: D4151E4; Nova-

clone; Dominion Biologicals Ltd., Dartmouth, Nova Scotia, Canada).

Genotyping and sequencing of RHD exons

DNA was isolated from EDTA-anticoagulated blood using commercial kits (DNA Extraction Kit magnetic beads, ZongJi WanTai Bio-pharmaceutical Co. Ltd., Jiangsu, China) based on automated Chemagic™ magnetic separation (FluoVia Gene Pure Plus; ZongJi WanTai Bio-pharmaceutical Co. Ltd.). All DNA samples were stored at -80°C until molecular analysis. The sequencing of exon 1–10 of the RHD gene and RHD genotype were analyzed using commercial kits (RHD fluo-genotyping and RHD exons sequence, ZongJi WanTai Bio-pharmaceutical Co. Ltd.).

RESULTS

Serological typing

The serological typing results are presented in **Table 1** and **Table 2**. The RHD phenotype in the proband was determined to be an RHD variant.

Table 1 Serological ABO and RHD typing in the proband

Subject	Anti-A	Anti-B	Anti-D (DVI)	Anti-D(DVI')	A cell	B cell	RHC	RHE	Screen I	Screen II	Screen III
Proband	0	4+	0	2+	4+	0	4+	4+	0	0	0
Wife	0	0	4+	4+	4+	4+	4+	4+	0	0	0
Daughter	0	4+	4+	4+	4+	0	4+	4+	0	0	0

Table 2 RHD confirmation test in the proband

Subject	Anti-D		Anti-D		Anti-D		clone: Absorption clone: Elution Test
	IgM clone: MS201	IgG clone: MS26	IgM clone: GAMA401	IgG clone: F8D8	IgM clone: D175-2	IgG clone: D4151E4	
Proband		2+		2+		2+	2+
Wife		4+		4+		4+	/
Daughter		4+		4+		4+	/

Note: The absorption elution test was based on acid elution and multiple polyclonal anti-D from RHD-pregnant women. The titer of anti-D was 1:128.

Molecular analysis of RHD gene

The RHD exon genotype contained exons 1 to 10, which primarily rule out the RHD and RHCE hybrid, leaving two RHD variant alleles. Molecular analysis of the proband demonstrated heterogenous single nucleotide polymorphisms consisting of 667 T>G, 676 G>C, 697 G>C, 712 G>A, 733 G>C, 744 C>T and 1227 G>A (**Fig.1**). The RHD allele in the proband's daughter was heterogeneous only for 1227 G>A, which also known as RHD*01EL.01. Pedigree analysis revealed 667 T>G, 676 G>C, 697 G>C, 712 G>A, 733 G>C and 744 C>T in the RHD allele, which was also an RHCE exon 5 sequence (**Fig.2**). This result

also concluded the allele, as an RHD-RHCE(5)-RHD hybrid, also called DVa-3 (RHD*05.03).

DISCUSSION

The D antigen is the most clinically significant antigen of the RH blood group system in clinical transfusion and obstetrics. There are many D variants, including weak D, partial D and Del^[6, 7]. In general, most D variant alleles are assumed to be generated by gene arrangements between RHD and RHCE genes, or by single missense mutations^[8]. However, it is very difficult to identify specific RHD alleles using serotyping. In this study, we used serotyping, genotyping,

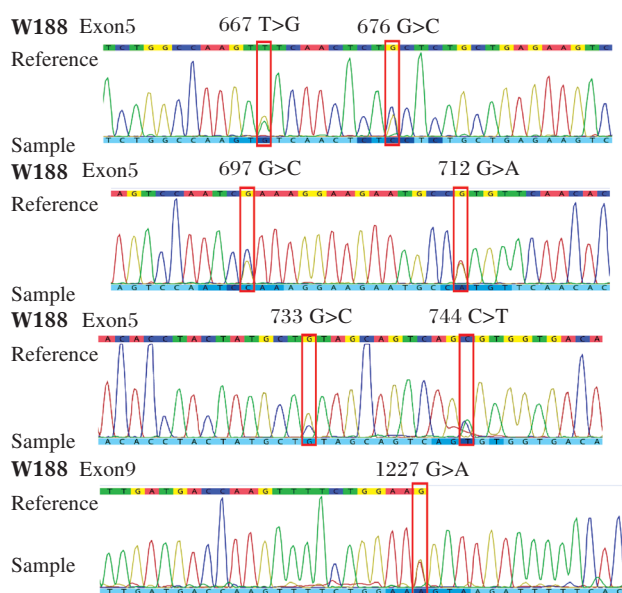


Fig. 1 RHD sequence analysis of the proband

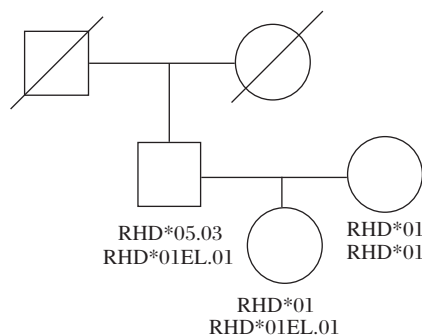


Fig. 2 Pedigree analysis for the RHD DVa allele

sequence and pedigree analysis to confirm the very rare (RHD*05.03) allele.

RHD sequence analysis of the proband revealed the following single nucleotide polymorphisms (SNPs): 667 T>G, 676 G>C, 697 G>C, 712 G>A, 733 G>C, 744 C>T and 1227 G>A. We have no information on the proband's parents, however, the daughter's RHD sequence demonstrated 1227 G>A. According to Mendel's Law of Inheritance, the novel RHD allele is 667 T>G, 676 G>C, 697 G>C, 712 G>A, 733 G>C, 744 C>T. However, as yet no RHD variant allele in concordant with the novel allele exists in the Erthyrogene database^[9]. Referring to RHCE and RHD reference alleles from BGMUT, 733 G>C and 744 C>T are SNPs for RHD and RHCE alleles; SNPs of 733 and 744 are the specific SNPs for RHD and RHCE (733C and 744T for RHCE; 733G and 744C for RHD), which means the SNPs are not associated with the variant allele^[10]. The alleles of 667 T>G, 676 G>C, 697 G>C, 712 G>A showed to be associated with RHD*05.03 (DV type 3). The RHD allele may belong to a new allele of DV because the novel allele

occurred in a variant in exon 5, while the RHD allele phenotype is consistent with DVI^[11]. The results also indicated that the use of simple monoclonal anti-D to identify RHD variant is inappropriate, because of the limited data on the molecular analysis of RHD variants in the Chinese population.

RHD genotyping of the proband's RHD genetic variants were reported to be 50%~70% RHD of the whole gene deficiency, 5.7% RHD-RHCE(2-9)-RHD hybrid and 20.2% 1227 G>A^[7]. Until now, over 40 types of RHD variant have been described in Chinese individuals and alloimmunisation to anti-D is a controversial topic^[12-18]. According to a report by Wang *et al.*, in RHD25 patients who received massive transfusions of RHD⁺ packed red blood cells, no anti-D immunisation was evident at days 168 and 232^[19]. Other reports found the period of anti-D alloimmunisation to be 60 to 180 days^[20-24]. Zhou *et al.* described anti-D immunisation in a patient with DBT-1 allele who received RHD⁺ packed red blood cells^[25]. Anti-D immunisation that occurs in people with RHD variants seems to be a complicated process. Due to the RHD phenotype showing negative in the 1227 G>A allele, the (RHD*05.03) phenotype is weak positive through multiple IgG/IgM mixed monoclonal anti-D. The accurate identification of RHD variant alleles in Chinese individuals may be a necessary strategy for clinical transfusion and for pregnant women.

In conclusion, the study demonstrated a very rare RHD DVa allele and indicated that the transfusion of RHD⁻ blood products was a useful strategy to avoid anti-D immunisation. However, the lack of epitope identity is a strong limitation. Further pedigree analyses should be conducted.

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