Transfusion and Apheresis Science 40 (2009) 169–173

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Sequencing analysis of *RHD* intron 7 and 9

Zhen Li, Chao-Peng Shao *

Shenzhen Blood Center and Shenzhen Institute of Transfusion Medicine, Meigang South Street, Nigang West Road, Futian, Shenzhen 518035, PR China

ARTICLE INFO

Keywords:
RHD
DEL
Intron
Sequence
Variants
Splicing site

ABSTRACT

Whole length of *RHD* introns 7 and 9 of one normal Rh D-positive individual and 2 DEL samples, carrying *RHD*1227A allele, were sequenced and aligned. Thirty-three and 27 nucleotide variants were totally observed in intron 7 and intron 9, respectively (EMBL/GenBank/DDBJ EU372940∼2). Among them, 8 variants in intron 7 and 7 in intron 9 were observed commonly in all 3 samples, whereas 2 variants in intron 7 and one in intron 9 were only found in 2 DEL samples, but not in the normal D-positive. The variants observed in intron 7 in DEL cannot explain enough for that DEL mRNA has one segment of 170 base pairs sequence from intron 7. But the nucleotides AG–GT at both sides of the segment may be related to this molecular even as AG–GT may cut intron 7 with its normal splicing site (GT–AG) into two "new introns" although the mechanisms are complicated in fact. We also have not found any suspicious splicing-affecting variants in intron 9 of DEL allele. However, this may make out further that the reason of whole exon 9 spliced out in DEL mRNAs may be no more than the 1227A>G mutation in DEL allele.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

The Rhesus blood group (Rh) is the most complex of the blood group systems. Rh antigens are encoded by two closely linked homologous genes, *RHD* and *RHCE*, encoding the antigens of D and CcEe, respectively. The D antigen (RH1) carried by the RhD polypeptide is of particular clinical importance with respect to transfusion- and pregnancy-induced allo-immunization. Numerous D variants have been characterized so far, both on the serologic and the molecular level [1,2]. Weak D variants express reduced numbers of D antigens as *RHD* point mutations with predicted amino acid substitutions. Partial D variants with characteristic D epitope loss are caused by either *RHD-CE-D* hybrid genes or *RHD* point mutations. The weakest D variants collectively called DEL appearing Rh-negative or D-negative even when anti-D typing is performed with a sensitive indirect antiglobulin test, and being serologically detectable only by adsorption–elution test [3]. But more data is still expected for molecular mechanisms of some types of DEL, for example DEL with *RHD*1227A allele [4,5].

Currently, throughout the world, the vast majority of DEL phenotypes are misinterpreted as D-negative owing to the limits of routine typing. But some data showed it was a questionable classification. In 2001, Feng et al. [6] reported a D-negative patient detected anti-D after transfusion of DEL (D^{el}) red blood cells in Chinese. In 2005, Yasuda et al. [7] observed an Rh-negative recipient produced allo-anti-D antibody with red cell transfusion from DEL donors carrying *RHD*1227A allele. Those studies documented the clinical significance of the DEL phenotype in blood units that was capable of inducing anti-D in a recipient. And it also makes us have to pay more attentions for DEL in transfusion- and pregnancy-induced allo-immunization, as well as in molecular understanding of this phenotype importantly.

Today at least seven kinds of DEL alleles have been found [4,8–12]. In the Chinese Han population, about 25–30% of Rh-negative are DEL [4,13,14], which is a very high rate compared to other ethnic populations. Furthermore, the current data show that the *RHD*1227A allele is a

* Corresponding author. Tel.: +86 755 83242567; fax: +86 755 83244188.
E-mail address: cpshaos@hotmail.com (C.-P. Shao).

1473-0502/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.transci.2009.03.004

dominating one in Chinese [4,13], called “Asian type” [15]. In 2006, our data revealed that the Asian type” DEL had at least 6 forms of mRNA (GenBank AY751491∼6), and among which 2 forms of mRNA contained 170 bp fragments from intron 7 (AY751495 and AY751496) [5]. More recently Richard et al. [12] observed the same event. But neither of these two studies explained its mechanism well. This study sequences whole length of intron 7 of the “Asian type” allele. In addition, the “Asian type” allele only has one synonymic mutation compared to normal *RHD* gene, which is G > A at the end of exon 9. Previous studies supposed the mutation might cause miss-splicing for intron 9. [4,9]. And our recent data indicates that all mRNAs of “Asian type” DEL lack the corresponding sequences of exon 9. It supports the exon 9 of DEL gene has been spliced always [12]. However, we are still interested in an investigation for any specific variants that occurred in intron 9, which may take part in this miss-splicing.

2. Materials and methods

2.1. Samples

One normal Rh-positive and 2 DEL samples, carrying *RHD*1227A allele, were selected for sequencing. All three samples have been carried out corresponding serological tests, *RHD* coding region sequencing analysis, *RHD* zygosity tests, and mRNA sequencing in our former studies [4,5,16–18]. The normal Rh-positive individual is *RHD* homozygote, represented as CCDDee, one DEL individual is *RHD* homozygote CCDDee, and another one is *RHD* heterozygote CcDdee.

2.2. Amplification and purification

Based on the *RHD/RHCE* sequences of GenBank BN000065, several pairs of specific oligonucleotide primers are designed via oligo 6.71 primer analysis software (Table 1). Whole intron 7 was amplified with 4 PCRs, and intron 9 was amplified with two fragments (Fig. 1). Total volume of each tube of PCR reaction is 50 μL, including DNA templet 2 μL (about 100 ng), $MgCl_2$ 1.25 mmol/L, dNTPs 200 μmol/L, high-fidelity DNA polymerase 2U (AmpliTaq Gold, Applied Biosystems, USA), and specific upper and lower primers. The conditions of PCR amplification (PE9700, Applied Biosystems company, USA) are pre-denaturation at 95 °C for 10 min, followed by thirty-five cycles of denaturation at 94 °C for 30 s, annealing at 60 °C or 63 °C for 30 s, and extension at 68 °C for 180 s, and 72 °C for 5 min at last. The PCR products are directly purified via commercial kits (Amicon Microcon-PCR Centrifugal Filter Devices, Amicon Bioseparations, Millipore, MA, USA), or gel was cut after electrophoresis separation and then purified through another kit (QIAquick Gel Extraction kit, Qiagen, Germany).

2.3. Sequencing and aligning

The most purified PCR products were taken to direct sequencing through BigDye™ Terminator Cycle and ABI Prism™ 3100 DNA Sequencer (Applied Biosystems, Foster

Table 1
PCR primer sequences, specificities and locations.

Primer	Sequence	3′-End	GenBank	Specificity	PCR/Tm
7U-1025	GGGTCTGCTTGGAGAGATCAT	*RHD* 1025	BN000065	*RHD*	PCR1/63 °C
7L-2538	GTATCTGCAACTGCATGATTCAG	INTRON 7-2538	BN000065	*RHD*	
7U-2471	GAGTCAGATGCTGTGATCAGAA	INTRON 7-2471	BN000065	*RHD*	PCR2/60 °C
7L-5184	GGCTCTCTGGGGTGGCT	INTRON 7-5184	BN000065	*RHD*	
7U-5154	GAGGGGGCATGCCTTCTT	INTRON 7-5154	BN000065	*RHD*	PCR3/63 °C
7L-7519	AGAAGGAAAACACCAAGTGTTG	INTRON 7-7519	BN000065	*RHD*	
7U-7448	GCTTTAGTGGCTACTGCTAAACAA	INTRON 7-7448	BN000065	*RHD*	PCR4/63 °C
8L-1105	CGATGGCCAAGCTGAGTTC	*RHD* 1105	BN000065	*RHD/CE*	
9-U1193	TAAAATATGGAAAGCACCTCATGA	*RHD* 1193	BN000065	*RHD*	PCR5/60 °C
9-L3597	CTTCGGAGGATGAGATGGGT	INTRON 9-3597	BN000065	*RHD/CE*	
9-U3540	TCAGCCACTGCCTCGCT	INTRON 9-0.3540	BN000065	*RHD/CE*	PCR6/60 °C
10-L+165	ATGGTGAGATTCTCCTCAAAGA	*RHD* +165	BN000065	*RHD*	

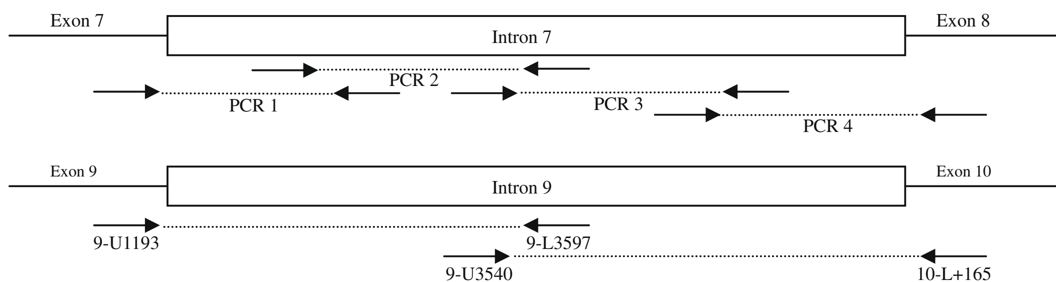

Fig. 1. Sketch for PCR amplification of *RHD* introns 7 and 9. The quadrate boxes represent *RHD* intron 7 or intron 9, the lines represent exons, the arrows and dashed lines represent PCR primers and amplification ranges.

Download English Version:

https://daneshyari.com/en/article/3335767

Download Persian Version:

https://daneshyari.com/article/3335767

Daneshyari.com