Available online at www.apjbg.com

Asia-Pacific Journal of Blood Types and Genes

2017, 1(3):29-32

APJBG

Which *RHD* alleles are risk factors stimulating allo–anti–D?

Fang Yan[1△], Yu–Shiang Lin[2,3△], Zhiyuan Xu[1], Xiaofei Li[1], Lei Zhang[1], Ye Zhang[1], Sufang Liu[1], Tianghong Miao[1*]

[1]*Blood Group Lab, Beijing Red Cross Blood Center, Beijing 100088, China;*

[2]*Department of Clinical Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China;*

[3]*College of Medicine, Aerospace Center Hospital, Beijing 100076, China*

ABSTRACT

The aim of this study was to identify the specific *RHD* alleles that are risk factors for stimulating allo–anti–D and develop a precise strategy for blood transfusion. To confirm the D phenotype, red blood cells suspended in saline should react to serological anti–D from three manufacturers. An antibody screen test, a saline phase test and a micro–column test were conducted to identify allo–anti–D and other allo–antibodies. *RHD* alleles were geno–typed by PCR using sequence–specific primers. Seven hundred subjects who were either pregnant or had under–gone transfusion were enrolled in our study; however, 28 samples were excluded because their *RHD* alleles were normal, as revealed by tests using genotyping kits. A total of 498 cases (74.1%) were RHD–*null* (lacking exons 1–10 of *RHD*), 336 were DEL *RHD1227A* (20.2%), and 38 were *RHD–CE*(2–9)–*D* (5.6%). There were 136 cases (20.2%) with allo–anti–D among the 672 cases, with an allo–anti–D prevalence of 126 cases (25.3%) in 498 cases that were *RHD–null*, followed by 10 cases (26.3%) among 38 cases with *RHD–CE*(2–9)–*D*, and none in 366 cases with *RHD1227A*. *RHD* genetic polymorphism was observed in RhD–negative individuals. We concluded that *RHD–null* and partial D are risk factors for alloimmunization to the D antigen and should be transfused with Rh–negative blood. *RHD1227A* recipients can be transfused with RhD–positive blood. Pregnant women with the d/d and *D–CE*(2–9)–*D* alleles require appropriate anti–D prophylaxis and *RHD1227A* may induce a higher tolerance.

Keywords: RhD–negative, anti–D, alloimmunization, partial D, Del

INTRODUCTION

Rh is the most complex erythrocytic antigen in the blood group system and presents the same clinical sta–tus as the ABO blood group system because antibod–ies against Rh antigens in certain circumstances lead to hemolytic disease in newborns and hemolytic transfusion reactions[1]. This can occur for example if RhD–negative pregnant women are immunized during pregnancy, re–sulting in the development of anti–D antibody.

The D antigen is the major immunogen of the Rh blood group system and its amino acid sequence sug–gests that Rh polypeptides traverse the membrane lipid bilayer 12 times[2,3]. The N and C terminal domains are both located inside the cell[2,4–7]. Weak D, partial D, and Del types can be identified using serological anti–D kits, and genotyping of *RHD* has revealed that there are more alleles for the serological D–negative type than for the positive type. However, few studies have systemically examined the seroprevalence of allo–im–munization for RhD antigen among the different *RHD* alleles in China. This data may provide support for a national transfusion policy and RhD immunoglobulin prophylaxis for RhD–negative pregnant women.

By statistically analyzing the results of tests con–ducted between 2013 and 2016, we determined the prevalence of allo–anti–D among *RHD* alleles. The

*Correspondence to: Tianhong Miao, Blood Group Lab, Beijing Red Cross Blood Center, Beijing 100088, China. TEL: +86–10–82807272; E–mail: tianhongm@163.com.

△These authors contributed equally to this work.

aim of this study was to identify specific *RHD* alleles as risk factors that stimulate allo−anti−D, in order to develop a precise strategy for blood transfusion.

MATERIALS AND METHODS

Study population

Blood samples collected into EDTA−containing tubes from 98 medical institutions in Beijing between March 2013 and June 2016 were sent to the Blood Group Laboratory (BGL) of the Beijing Red Cross Blood Center (BRCBC). The initial inclusion criterion was women who had a history of pregnancy or underwent transfusion, and the exclusion criterion was subjects whose natural immune allo−anti−D or D alleles could not be detected.

For each sample received, the BRCBC first performed ABO Rh typing, and unexpected antibody screening by conventional tube techniques at room temperature and gel column test simultaneously. Seven hundred patients with serological D−negativity or weak positivity (serological titer under 2+) were enrolled in our study, from which 28 samples were excluded because their *RHD* alleles were normal alleles, as revealed by tests using genotyping kits. Forty−four patients had transfusion history and 628 patients had pregnancy history.

Phenotyping of RhD, and antibody screening and identification

Three different IgG/IgM mixed monoclonal antibodies against RhD antigen from DBL(Canada), Millipore(UK), and CLAS(UK) were used to identify the Rh variants. The screening and identification cells used at the BGL of the BRCBC cover many antigen systems, including Rh, MNS, Duffy, Kidd, Kell, Lewis, P1, Xg, and Lutheran, as well as low−frequency antigens such as Dia and Mur (Bio−Rad, Hercules, CA, USA and Sanquin, Amsterdam, Netherlands), which have a relatively high prevalence in Asia. Detection methods included conventional tube techniques conducted at room temperature and gel column tests (Bio−Rad).

DNA extraction and RHD genotyping

DNA was isolated from EDTA−anti−coagulated blood, using a commercial kit (Prepito DNA Blood 250 Kit, Chemagen, Perkin−Elmer, Waltham, MA, USA) based on magnetic separation in an automated system (Chemagic Prepito, Chemagen, Perkin−Elmer, USA). Polymerase chain reaction with sequence−specific primers (PCR−SSP) was performed for RhD blood group genotyping using commercial kits (*RHD−negative* genotype, Screen, Jiangsu LiBioMedicine Biotechnology, China; *RHD* genotyping, Biosuper, Tianjing, China). These two kits were used to genotype the major *RHD−negative* alleles, including *RHD−null*, *D−CE*(2−9)*−D*, *weak D15*, *RHD1227A*, and *RHD−negative* genotypes (Screen). *RHD* exons 1−7, 9, and 10 were further analyzed to screen for the types of exon deficiency. PCR amplification products of *RHD* genotyping were analyzed by DNA electrophoresis and examined under UV light. *RHD−negative* genotypes (Screen) were subjected to real−time PCR using Sybr Green Ⅰ and then the melting curve and melting temperature were analyzed.

RESULTS

Seroprevalence of allo−anti−D and pregnancy as risk factors

A total of 192 pregnant women recorded pregnancies as G2P0, followed by 41 pregnant women with G2P1, 125 with G3P0, and 38 with G3P1, and 38 women experienced pregnancy more than 3 times; 44 patients underwent transfusion.

Upon evaluating whether the frequency of pregnancy was a risk factor, the number of pregnant women with G2P0 compared to those with G3P0 was insignificant, followed by G2P1 (OR: 3.51; 95%CI: 1.49−8.27), G3P1 (OR: 8.84; 95%CI: 4.22−18.52), and multi pregnancies (OR: 20.57; 95%CI: 10.27−41.22) ***(Table 1)***.

Table 1 **Seroprevalence of all−anti−D among women with transfusion and pregnancy**

Immune factors	Allo−anti−D/*n*(%)	OR	95%CI
Transfusion (*n*=44)	44(100)	−	−
Pregnancy			
G2P0 (*n*=192)	8(4.2)	Reference	Reference
G3P0 (*n*=250)	20(8.0)	1.92	0.86−4.27
G2P1 (*n*=82)	12(14.6)	3.51	1.49−8.27
G3P1 (*n*=76)	28(36.8)	8.84	4.22−18.52
More (*n*=28)	24(85.7)	20.57	10.27−41.22

Seroprevalence of allo−anti−D among pregnant women with different *RHD* genotypes

Genotyping of *RHD* revealed 498 pregnant women with *d/d* (74.1%), 38 pregnant women with *RHD−CE*(2−9)*−D*(5.7%), and 136 pregnant women with *RHD1227A*(20.2%). The seroprevalence of allo−anti−D was 25.3% for the *d/d* genotype, 26.3% for the *RHD−CE*(2−9)*−D* genotype, and none for allo−anti−D in *RhD1227A*.

DISCUSSION

Production of alloantibodies against Rh antigens is an important issue and mirrors the ABO system in clinical transfusion; RhD is the major immunogen between Rh antigens. In contrast to Caucasian or other ethnicities, Chinese people harbor polymorphisms in *RHD* alleles, most frequently *RHD−null* (approximately 20%), followed by *RHD1227A* alleles (approximately 5%~6%). Also in contrast to that in Caucasian or other ethnicities, *RHD−null* was the most frequent *RHD* allele among negative phenotypes[8−9]. Our study demonstrated that pregnant women with *d/d* or *D−CE*(2−9)−*D* genotypes were sensitized to alloimmunization of RhD through transfusion or pregnancy, but *RHD1227A* was not a factor in allo−anti−D production.

Although pregnancy and transfusion are major risk factors in the alloimmunization of red blood cell antigen, few studies have compared the frequency of gravida− para− abortion (GPA) immune allo−anti−D [10−11]. We found that 628 pregnant women who experienced pregnancy many times had a sero−prevalence of 4.2% in G2P0 as a reference, 8.0% in G3P0, 14.6% in G2P1, and 36.8% in G3P1. This result demonstrates that for the allo−anti−D immunization, the frequency of GPA and parturient are the highest immune factor aside from abortion, as G2P0 compared to G3P0 did not significantly stimulate allo−anti−D.

The *d/d* and *D−CE*(2−9)−*D* alleles were immunized by transfusion and/or pregnancy to produce allo−anti−D. Because *D−CE*(2−9)−*D* lacks *RHD* exons 1−10, lower levels of D antigen are expressed on the red blood cell membrane and the alleles provided immunity to the D antigen. Our study revealed an allo−anti−D seroprevalence of 25.3% in *d/d* and 26.3% in *D−CE*(2−9)−*D*, suggesting that in patients with *D−CE*(2−9)−*D*, transfusion with D null red blood cells is necessary. Additionally, 10%−30% of subjects with *RHD1227A* were serological D−negative and the alleles were homozygous for a G>A mutation at the final base of exon 9 in the *RHD* gene, resulting in an RhD splicing site error[12,13]. For the RhD epitope on the red blood cell membrane of *RHD1227A*, Zhu *et al*. quantified the D epitope using fluorescent−labeled anti−D by flow cytometry and the D epitope was found to be lower than the flow cytometry detecting limit (<22 epitopes per red blood cell). Further, our results agree with those of previous studies showing that no pregnant women who were immunized produced allo−anti−D[14]. Therefore, subjects with *RHD1227A* may be strongly tolerant to transfusion with D antigen positive red blood cell products to save RhD−negative red blood cells. In contrast to *RHD1227A* recipients, several studies published in China, Japan, and Europe reported that patients with RhD−null blood produce allo−anti−D following transfusion from *RHD1227A* donors[15−16]. However, it remains controversial whether *RHD1227A* from RhD−positive red blood cells can be used for transfusion.

We found that RhD−null and partial D pose a risk of alloimmunization to the D antigen and should be transfused with Rh−negative blood. *RHD1227A* recipients can be transfused with RhD−positive blood. Pregnant women with the *d/d* and *D−CE*(2−9)−*D* alleles require appropriate anti−D prophylaxis and *RHD1227A* may have a higher tolerance.

References

[1] Westhoff CM. The Rh blood group system in review: a new face for the next decade. *Transfusion*, 2004, 44: 1663−73.

[2] Avent ND, Ridgwell K, Tanner MJA, et al. cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (Rhesus)−blood−group−antigen expression. *Biochem J*, 1990, 271: 821−5.

[3] Kajii E, Umenishi F, Iwamoto S, et al. Isolation of a new cDNA clone encoding an Rh polypeptide associated with the Rh blood group system. *Hum Genet*, 1993, 91: 157−62.

[4] Chérif−Zahar B, Bloy C, Le Van Kim C, et al. Molecular cloning and protein structure of a human blood group Rh polypeptide. *Proc Natl Acad Sci USA*, 1990, 87: 6243−7.

[5] Hermand P, Mouro I, Huet M, et al. Immunochemical characterization of Rhesus proteins with antibodies raised against synthetic peptides. *Blood*, 1993, 82: 669−76.

[6] Avent ND, Butcher SK, Liu W, et al. Localization of the C termini of the Rh (Rhesus) polypeptides to the cytoplasmic face of the human erythrocyte membrane. *J Biol Chem,* 1992, 267: 15134−9.

[7] Eyers SAC, Ridgwell K, Mawby WJ, et al. Topology and organization of human Rh (Rhesus) blood group −related polypeptides. *J Biol Chem,* 1994, 269: 6417−23.

[8] Wagner FF, Moulds JM, Tounkara A. RHD allele distribution in Africans of Mali. *BMC Genet,* 2003, 24(4):14.

[9] Rodrigues A, Rios M, Pellegrino J Jr. Presence of the RHD pseudogene and the hybrid RHD−CE−D(s) gene in Brazilians with the D−negative phenotype. *Braz J Med Biol Res,* 2002,35(7):767−73.

[10] Karim F, Moiz B, Kamran N. Risk of maternal alloimmunization in Southern Pakistan − a study in a cohort of 1000 pregnant women. *Transfus Apher Sci*, 2015,52(1):99−102.

[11] Xu P, Li Y, Yu H. Prevalence, specificity and risk of red blood cell alloantibodies among hospitalised Hubei Han Chinese patients. *Blood Transfus*, 2014,12(1):56−60.

[12] Shao CP, Maas JH, Su YQ, et al. Molecular background of RhD–positive, D–negative, Del and weak D phenotypes in Chinese. *Vox Sang*, 2002,83(2):156–61.

[13] Kim JY, Kim SY, Kim CA, et al. Molecular characterization of D–Korean persons: development of diagnostic strategy. *Transfusion*, 2005,45(3):345–52.

[14] Chu M, Zhou D, Xie Y. Quantification of D epiyope on red cell membrane of patients with RhD 1227A. *Chinese Journal of Blood Transfusion(In Chinese)*, 2008,21(7):512–3.

[15] Wanger T, Kormoczi GF, Buchta C, et al. Anti–D immunization by Del red blood cells. *Transfusion*, 2005,45(4):520–6.

[16] Yasuda H, Ohto H, Sakuma S, et al. Secondary anti–D immunization by Del red blood cells. *Transfusion*, 2005,45(10):1581–4.

(Received 03 September 2017, Revised 07 September 2017, Accepted 12 September 2017)