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The *RHD* variants in Chinese population

Ming Gao*, Yuping Chen

Tim Hunt Nobel Prize Institute, Jiangsu LIBO Medicine Biotechnology Co., Ltd. Jiangyin, Jiangsu, 214400, China.

ABSTRACT

The Rhesus (Rh) blood group system is the most important blood group system in hemolytic disease of the fetus and newborn (HDFN). In clinical transfusions, the D antigen in the Rh blood group system comes third, behind antigens A and B which from ABO blood group system. Over the past decade, molecular technologies have been used to investigate the *RHD* allele in different ethnic groups. This review first introduces the basic structure of RhD protein and coding genes, then focuses on D-negative, weak D, partial D, DEL, RhD_{null} variants reported in the Chinese population. To date, more than 460 *RHD* variants have been reported around the world, but less than 70 *RHD* variants have been reported in the Chinese population. Further research is needed to identify more *RHD* polymorphism and establish criteria for blood detection and transfusion guidelines for *RHD* variants. Only in this way can we better guarantee the safety of blood transfusion and prevent the occurrence of HDFN. With the accumulation of research and clinical data, we should be clearer which *RHD* variants are to be regarded as RhD negative and which need to be regarded as RhD positive.

Keywords: *RHD*, Rhesus blood group, D variant, polymorphism

INTRODUCTION

The Rhesus (Rh) blood group system is the most important blood group system in hemolytic disease of the fetus and newborn (HDFN). In clinical transfusions, the relevance of D antigen in the Rh blood group system comes third, behind antigens A and B which from ABO blood group system. Over the past decade, molecular technologies in combination with serological testing have been used to investigate the *RHD* allele, which encodes D antigen in different ethnic groups. As a consequence, many D antigen variants and *RHD* alleles have been observed and described[1]. According to D antigen density and epitopes serologically, D can be classified into D-positive, weak D, partial D, DEL, and D-negative. *RHD* alleles for these phenotypes are extremely complex. DNA sequencing has revealed the complexity of the Rh blood group system in which a vast array of *RHD* variant alleles exist, such as mutations, deletions, conversions, or insertions, which are observed in the coding or non-coding regions by comparing these sequences with the *RHD* sequence from a normal D-positive individual. To date, more than 460 *RHD* alleles have been registered and categorized[2–4]. When compared to the hundreds of *RHD* variant polymorphisms in Caucasians, *RHD* variants in Chinese population are much fewer. This review will address the progress that *RHD* polymorphism analyses have made in the Chinese population.

THE BASIC STRUCTURE OF RhD ANTIGENS

Rh proteins are expressed only in mature red blood cells (RBCs) and erythroid progenitors at the colony-

*Correspondence to: Ming Gao, Tim Hunt Nobel Prize Institute, Jiangsu LIBO Medicine Biotechnology Co., Ltd. Jiangyin, Jiangsu 214400, China. E–mail: 6603gaoming@163.com.

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forming unit-erythroid (CFU-E) stage at 100~200,000 copies per cell. RhD and RhCE proteins are not phos–phorylated or glycosylated, but palmitoylated mem–brane proteins of 417 amino acids are predicted to be organized in 12 transmembrane α-helix domains[5]. This multi-pass transmembrane protein is composed of 6 extracellular domains and 7 intracellular domains (***Fig. 1***)[6].

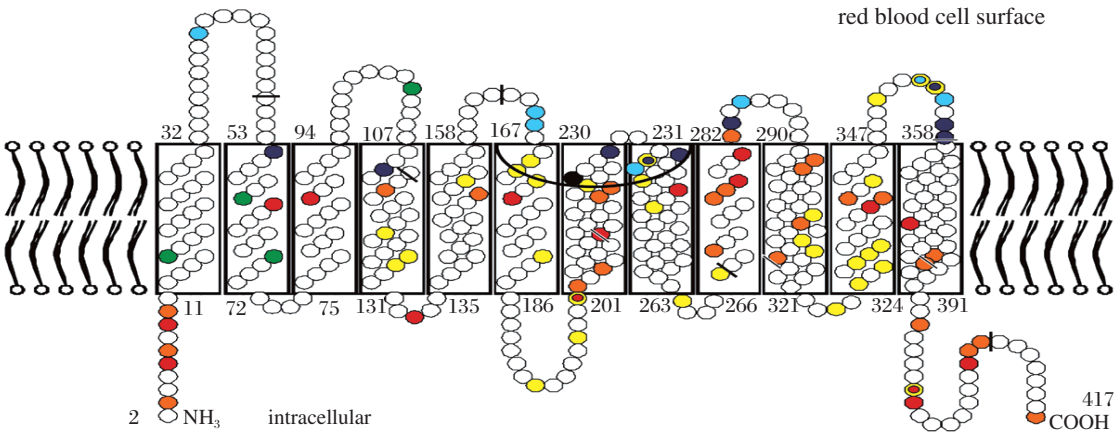

Fig. 1 **Model of Rhesus proteins in the red blood cell membrane.** Both Rhesus proteins comprise 417 amino acids, shown here as circles. Mature proteins in the membrane lack the first amino acid. The amino acid substitutions that distinguish the RhCE from the RhD protein are marked in yellow, with the four amino acids that code for the C antigen in green and the one that codes for the E antigen in black. The single amino acid substitutions which code for partial D are in blue, and those that code for weak D are in red. The extracellular Rh vestibule is depicted by the inverted black arc and bordered in part by amino acids of loops 3 and 4. The nine exon boundaries in the *RHD* cDNA, as reflected in the amino acid sequence, are indicated by black bars[6]. (Reproduced by permission from Flegel WA. Molecular genetics and clinical applications for RH. *Transfus Apher Sci*, 2011, 44: 81–91.)

THE MOLECULAR BASIS OF Rh PHENO–TYPES

The *RH* locus on chromosome 1p34-p36 is com–posed of homologous *RHD* and *RHCE* genes (96% identity), which are tandemly organized in opposite orientation and interspersed by a third gene, *SMP*1, whose function is presently unknown. The *RHD* gene is flanked by two repeated sequences (9,000 bp) ex–hibiting 98.6% homology known as "rhesus boxes" (***Fig. 2***)[5].

Besides *RHD* deletion causing a D negative pheno–type, a host of RhD protein variants expresses altered D antigens. There is no absolute correlation between the molecular structures, phenotypes and clinical rel–evance of *RHD* alleles. To provide some degree of order to a large number of aberrant D antigens, *RHD* alleles are classified according to their phenotype and molecular variation in partial D, weak D, DEL, and RhD_{null} (***Table 1***)[6].

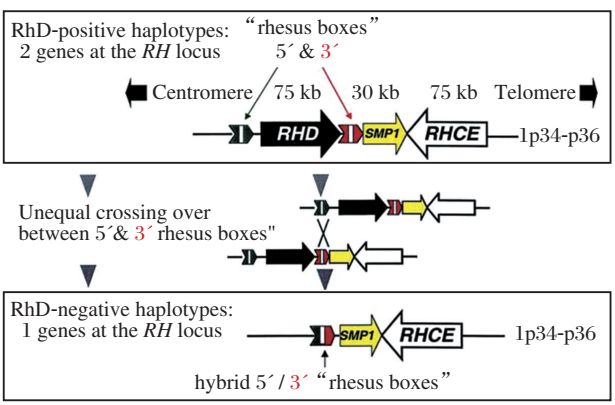

Fig. 2 **RhD-positive/RhD-negative polymorphism.** In RhD-positive haplotypes, the *RH* locus is composed of the *RHD* and *RHCE* genes in the opposite orientation, whereas the *RHD* gene is absent from RhD-negative haplotypes. The presence of a hybrid 5′/3′rhesus box in RhD-negative locus strongly suggests that *RHD* gene deletion occurred by unequal crossing-over between 5′ and 3′rhesus box of two RhD-posi–tive chromosomes[5]. (Reproduced by permission from Kim CL, Colin Y, Cartron JP. Rh proteins: key structural and functional components of the red cell membrane. *Blood Rev*, 2006, 20: 93–110.)

D-NEGATIVE IN CHINESE POPULATION

The D-negative phenotype shows wide racial dif–ferences with respect to its frequency; occurring in approximately 15% of Caucasians, 3% to 7% of Af–ricans, and less than 1% of persons from the Far East. In Chinese individuals, the corresponding frequency is approximately 0.2% to 0.4%. From a total of 890,403 blood donors investigated in Northwest China, Shanxi, Ye SH *et al*. reported 2,493(0.28%) D-negative sam–ples, with *RHD* deletion assessed as 1,685 (67.59%) among serologically D-negative donors[7]. Zhang X *et al*. investigated 132,479 blood donor samples in Northeast China, finding 495 (0.347%) to be D-neg–ative serologically[8]. Ye L *et al*. investigated 300,000 blood donors in Shanghai, from which D-negative

Table 1 **Representative molecular changes in *RHD* alleles expressing distinct phenotypes of the D antigen**

Classification of antigen variation	D antigen phenotype	Molecular basis		Representation example	
		Protein variation	Mechanisms	RHD allele	Trivial name
Partial D	Qualitative change	Amino acid substitution on the RBC surface	Missense mutation	*RHD*(G355S)	DNB
		Protein segment exchange on the RBC surface	Gene conversion (hybrid protein)	*RHD-CE*(3-6)-*D*	DVI type 3
Weak D	Quantitative change	Amino acid substitution in the membrane or intracellularly	Missense mutation	*RHD*(V270G)	Weak D type 1
DEL	Major quantitative change	Grossly reduced translation or protein expression	Missense mutation	*RHD*(1227G>A)	Not applicable
			Mutation at splice site		
D-negtive	D-negtive	Lack of protein expression	Gene deletion	*RHD*-Deletion	D negative
			Nonsence mutation	*RHD*(Y330X)	Not applicable
			Frame shift mutation	*RHD*(488del4)	Not applicable
		Protein segment exchange on the RBC surface	Modifying gene	Defect of *RHAG* gene	Rh_{null}
			Gene convertion (hybrid protein)	*RHD−CE*(3-7)-*D*	Cde

donors were 722 (0.24%)[9]. With the addition of 11 D-negative samples from Northern China, Inner Mongolia Autonomous Region, all 733 D-negative samples were confirmed by *RHD* exon specific PCR. This resulted in a total of 632 (86%) samples testing negative, suggesting homozygosity for the *RHD* deletion (d/d). An investigation in Xinjiang revealed 150 D-negative results serologically from 15,643 blood donors, from which, according to identification records, the D−negative donors fell among 86 Uyghurs (86/1,814, 4.74%), 50 Hans (50/12,546, 0.40%), 7 Huis (7/695, 1.01%), 4 Kazaks (4/157, 2.55%), 2 Mongols (2/397, 0.5%) and 1 Khalkha (1/34, 2.9%), respectively[10]. Another study in Xinjiang among 194,812 blood donors reported 1,390 D-negative samples, comprising of 634 Hans (0.38%) and 756 other Minorities (2.85%), respectively[11]. Chen W *et al*. analyzed in patients from Xinjiang Uyghur Autonomous People´s Hospital and the Traditional Chinese Medicine Hospital of Xinjiang Medical University, and demonstrated RhD negative frequencies of 0.35%(2/574) in Han, 2.7%(8/294) in Uyghur and 3.6%(1/28) in Kazak patients respectively[12]. A larger survey in Shanghai showed 1,237(0.31%) D-negative results among 400,253 blood donors[13]. The homozygote for missing *RHD* coding genes accounted for about 0.19% of the Han population in Northwest China, 0.25% of the Han population in Northeast China, 0.21% in East China, and 63.5% of the Han Rh serologically negative people in South China[7−11,14]. The most frequent D negative haplotype in humans is due to *RHD* deletion. Other important D negative alleles are the *RHD* pseudo-gene *RHD*ψ and the *RHD-CE-D* hybrid allele. Besides these, point mutations, where by an insertion of the nucleotide segment into *RHD* genes causes the termination of translation, known as non-functional alleles. More than 90 negative D genotypes have been identified in the Rhesus base so far. The most common non-functional *RHD* alleles found in the Chinese population are *RHD*01N.03 [hybrid RHD*D−CE(2-9)−D] and RHD*01N.16 (RHD*711delC).*

WEAK D IN CHINESE POPULATION

Weak D was first reported as as D variant antigen in 1946 by Stratton, finding that if an amino acid substitution was located in the transmembrane or intracellular segments of the RhD protein (***Fig. 1***), a weak D phenotype may occur[4−6]. There are four main requirements when assigning a weak D type:

(1) The *RHD* allele must be defined by nucleotide sequencing of the full-length *RHD* coding sequence, either as cDNA or from genomic DNA covering all 10 *RHD* exons.

(2) At least one amino acid substitution must be located in the membrane or below the membrane of the RBC.

(3) Proof of a weak D phenotype must be confirmed by peer-approved method. The preferred determination is the D antigen density of less than about 5,000 antigens per RBC using the flow cytometry method. As an alternative, weak reactivity by standard serology with monoclonal antibodies of IgM or IgG type in tube or gel is also acceptable.

(4) The deposition of the *RHD* allele in the nucleotide sequence database[2].

Currently, 172 weak D types have been identified in the Rhesus base. Thirty-five weak D types have been reported in the Chinese population (***Table 2***). Among these weak D variants, *RHD**weak D type 15 is the most prevalent, attaining 22.7% (17/75) of allele frequency among D variants in South China, Guangzhou, and 34.4% (11/32) of D variants in East China, Zhejiang, respectively[14,15]. Considering that the number of laboratories capable of genotyping and sequence analysis in China is less than ideal, the number of weak D types in the Chinese population is believed to be under

***Table 2 RHD* alleles found in Chinese with a serologic weak D phenotype**

Designation	Membrane localization	Mutation position	Alterations	Mechanism	ISBT terminology	References
Weak D type 1	TM 9	Extron 6	c.809 T>G p.V270G	Single missense mutation	*RHD**01W.1 *RHD**weak D type 1	Found only in Taiwan[13]
Weak D type 6	IC 1	Extron 1	c.29 G>Ap.R10Q	Single missense mutation	*RHD**01W.6 *RHD**weak D type 6	Found only in Taiwan[14]
Weak D type 12	TM 9	Extron 6	c.830G>Ap.G277E	Single missense mutation	*RHD**01W.12 *RHD**weak D type 12	[14]
Weak D type 15	TM 9	Extron 6	c.845G>A p.G282D	Single missense mutation	*RHD**15 *RHD**weak partial 15	
Weak D type 18	IC 1	Extron 1	c.19C>T p.R7W	Single missense mutation	*RHD**01W.18 *RHD**weak D type 18	
Weak D type 24	TM 11	Extron 7	c.1013T>Cp.L338P	Single missense mutation	*RHD**01W.24 *RHD**weak D type 24	[15]
Weak D type 25	TM 4	Extron 3	c.341G>A p.R114Q	Single missense mutation	*RHD**01W.25 *RHD**weak D type 25	
Weak D type 31	IC 1	Extron 1	c.17C>T p.P6L	Single missense mutation	*RHD**01*W*.31 *RHD**weak D type 31	
Weak D type 33	TM 6	Extron 4	c.520G>A p.V174M	Single missense mutation	*RHD**01W.33 *RHD**weak D type 33	
Weak D type 51	IC 4	Extron 1	Hybrid RHCE (4:594–4:602)	Hybrid allele	*RHD**01W.51 *RHD**weak D type 51	[16]
Weak D type 52	TM 1	Extron 1	c.92T>Cp.F31S	Single missense mutation	*RHD**01W.52 *RHD**weak D type 52	[16]
Weak D type 53	TM 8	Extron 5	c.740T>Gp.V247G	Single missense mutation	*RHD**01W.53 *RHD**weak D type 53	[16]
Weak D type 54	TM 4	Extron 3	c.365C>T p.S122L	Single missense mutation	*RHD**01W.54 *RHD**weak D type 54	
Weak D type 59	IC 1	Extron 8	c.1148T>C p.L383P	Single missense mutation	*RHD**01W.59 *RHD**weak D type 59	[17]
Weak D type 71	IC 1	Extron 1	c.29 G>Cp.R10P	Single missense mutation	*RHD**01W.71 *RHD**weak D type 71	[18]
Weak D type 72	IC 7	Extron 9	c.1212C>A p.D404E	Single missense mutation	*RHD**01W.72 *RHD**weak D type 72	
Weak D type 73	IC 7	Extron 10	c.1241C>TA414V	Single missense mutation	*RHD** 01W.73 *RHD**weak D type 73	[19]
RHD(IVS3+3G > C)	/	IVS3+3	IVS3+3G>C	Splicing site	/	[19]
Weak *RHD* (IVS6-14delTAA)	/	IVS6−14	IVS6−14del3,	Splicing site	/	[18]
RHD(IVS4+5G > A)	/	IVS4+5	IVS4+5G>A	Splicing site	/	[19]
RHD(IVS4+5G > T)	/	IVS4+5	IVS4+5G>T	Splicing site	*RHD**01EL.14 *RHD**DEL14	[19]
Weak D type 95	TM 8	Extron 5	c.730G>C p. A244P	Single missense mutation	*RHD** 01W.95 *RHD**weak D type 95	
RHD (G255R)	TM 8	Extron 5	c.763G>C p.G255R	Single missense mutation	/	
RHD (Y34C)	EF 1	Extron 1	c.101A>G p.Y34C	Single missense mutation	/	
weak D type 37	IC 3	Extron 3	c.399G>C p.K133N	Single missense mutation	*RHD**01W.37 *RHD**weak D type 37	
weak D type 149	IC 5	Extron 5	c.779A>G p.H260R	Single missense mutation	/	
*RHD*1252G	IC 7	Extron 10	c.1252T>G p.X418E			[20]
RHD(I341N)	TM 11	Extron 8	c.1022T>A p.G368R	Single missense mutation	/	[21,23,24]
DBO2	TM 4	Extron 3	c.357T>C	Silent mutation	/	[22]
weak D type 146	TM 5	Extron 3	c.438G>Cp.E146D	Single missense mutation	/	[22]
weak *RHD*(960G>A)	IC 6	Extron 7	c.960G>A p.L320L	Silent mutation	/	[23]
weak D type 100	IC 5	Extron 5	c.787G>A p.G263R	Missense (splice site affected)	*RHD** 01W.100 *RHD**weak D type 100	[24]
weak D type 147	TM 5	Extron 3	c.436G>C p.E146K	Single missense mutation	/	
RHD(T32N)	TM 1	Extron 1	c.95C>A p.T32N	Single missense mutation	/	[25]
weak D type 148	TM 7	Extron 5	c.670A>G N224D	Single missense mutation	/	[26]

Three types of membrane localization of amino acid substitution, IC (intracellular), EF (exofacial), and TM (transmembraneous) were predicted according to the model for orientation of the RhD protein proposed by Wagner and coworkers. /: no ISBT teminology.

reported. Therefore, further investigation is needed to identify new weak D variants in Chinese people.

PARTIAL D IN CHINESE POPULATION

In 1984, Salmon first introduced the term "partial D"[16]. If an amino acid substitution is located in an extracellular loop (***Fig. 1***), a partial D phenotype may result. Many new partial D types were found since 2000 and it is likely many more will be discovered. Partial D is of clinical relevance because carriers may produce anti-D upon exposure to the normal D antigen. To date, there are 119 partial D types listed by the Rhesus database (http://www.Rhesus base.info/). It should also be noted that proof of anti-D immunization is not a prerequisite for labeling a partial D allele. There are many partial D phenotypes for which no anti-D immunization event has been documented so far. The distinction between weak D and partial D alleles is further compounded by the fact that several partial D phenotypes, including the clinically important DVI, express their D antigen weakly[17]. *RHD*DVI.3* is the most widely distributed partial D phenotype in the Chinese population, attaining 38.7% (29/75) of allele frequencies in Guangzhou among D variants alleles[14], and 68.2% (30/44) in East China, Shanghai. A study including 1,274,540 blood donors of most blood types, demonstrated the incidence of partial D in the Chinese population to be approximately 0.003%, based on screening of blood donors in Shanghai[18]. Partial D literature reported in the Chinese population is summarized in ***Table 3***.

DEL IN CHINESE POPULATION

In 1984, Okubo and colleagues reported their observation that "some D-negative red cells, though they were negative in a Du test after exposure to anti-D, could bind anti-D and yield it on elution." They named these red blood cells´ phenotype Del (D eluate)[19]. During the subsequent 30 years, these observations have been confirmed by several investigators. The Del phenotype has been renamed DEL and is, in D–negative East

***Table 3 RHD* alleles found in Chinese with a serologic partial D phenotype**

Designation	Membrane localization	Mutation position	Alterations	Mechanism	ISBT terminology	References
DV type 2	IC, TM, EF	Extron 5	Hybrid *RHCE*(5)	Hybrid allele	*RHD**05.02 *RHD**DV.2	[8]
DVI type 3	IC, TM, EF	Extron 5	Hybrid *RHCE*(3–6)	Hybrid allele	*RHD**06.03 *RHD**DVI.3	[8]
DVI type 4	IC, TM, EF	Extron 5	Hybrid *RHCE*(3–5)	Hybrid allele	*RHD**06.04 *RHD**DVI.4	[8]
DHK	EF	Extron 5	697G>A (E233K)	Single missense mutation	*RHD**05.05 *RHD**DV.5	[30]
DBT–1	IC, TM, EF	Extron 5	Hybrid *RHCE*(5–7)	Hybrid allele	*RHD**14.01 *RHD**DBT1	[30]
DFR type1	TM	Extron 4	Hybrid *RHCE*(4:505–4:514)	Hybrid allele	*RHD**17.01 *RHD**DFR1	[30]
DFR type2	IC, TM, EF	Extron 4	Hybrid *RHCE*(4)	Hybrid allele	*RHD**17.02 *RHD**DFR2	[8]
DFR type4	TM	Extron 4	Hybrid RHCE(4:505–4:509)	Hybrid allele	*RHD**17.04 *RHD**DFR4	[30]
DLX	IC, TM, EF	Extron 5	667T>G (F223V) Hybrid *RHCE*(5:712–6)	Complex changes	*RHD**46 *RHD**DLX	[30]
DCC	TM, EF	Extron 5	677C>A (A226D)	Single missense mutation	*RHD**42 *RHD**DCC	[30]
DCS–1	TM, EF	Extron 5	667T>G (F223V) 676G>C (A226P)	Hybrid allele	*RHD**16.01 *RHD**DCS1	[25]
DCS–2	TM, EF	Extron 5	676G>C (A226P)	Single missense mutation	*RHD**16.02 *RHD**DCS2	[30]
DIVb type 2	IC, TM, EF	Extron 5	Hybrid *RHCE*(7:1048–9)	Hybrid allele	*RHD**04.06 *RHD** DIVb	[31]
DLO	EF	Extron 6	851C>T (S284L)	Single missense mutation	*RHD**36 *RHD**DLO	[32]
DV type 1	IC, TM, EF	Exon 5	Hybrid *RHCE*(5:667–5:697)	Hybrid allele	*RHD**05.01 *RHD**DV.1	[29]
DV type 2	IC, TM, EF	Exon 5	Hybrid *RHCE*(5)	Hybrid allele	*RHD**05.02 *RHD**DV.2	[29]
DV type 8	TM	Exon 5	Hybrid *RHCE*(5:667–5:744)	Hybrid allele	*RHD**05.08 *RHD**DV.8	[29]

Asians, predominantly caused by the *RHD*(1227G>A) allele, which has since been termed *RHD*DEL1 or RHD*01EL.01* (International Society of Blood Transfusion[ISBT] terminology). The *RHD*DEL1* is the most prevalent variant *RHD* allele, and *RHD*DEL2* (*RHD* 3G>A) is thought to be the second most common *DEL* allele, at least in Chinese populations[13,20]. The prevalence of DEL phenotypes is 30 percent for native Chinese and 32 percent for those living in Taiwan[21,22]. The *RHD*DEL*1 allele, which accounts for up to in serologically negative people. 98 percent of DEL phenotypes in East Asians, was also termed as "Asia type DEL" by Shao *et al*.[22–24]. To date, investigators have identified more than 45 alleles that are associated with the DEL phenotype. *DEL* phenotypes in the Chinese population published to date are summarized in ***Table 4***.

Shao suggests that persons in East Asian populations who carry the DEL variants can safely receive transfusions from D-positive donors. However, carriers of DEL can still be D-negative donors, since there are fewer than 22 membrane RhD antigens per DEL RBC, as compared with thousands of RhD antigens on a normal RhD-positive RBC[23]. Unfortunately, allo–immunization following transfusion of DEL blood by D-negative recipients has been reported[25–27]. Therefore, more researches and clinical data accumulation are needed to clarify DEL serological characteristics.

***Table 4 RHD* alleles found in Chinese with DEL phenotype**

Designation	Membrane localization	Mutation position	Alterations	Mechanism	ISBT terminology	References
RHD(1227G > A)	IC	Exon 9	1227G>A	Splice site mutation	*RHD**01EL.01 *RHD**DEL1	[38]
RHD-RHCE(2-5)-RHD	IC, TM, EF	Exon 2–5	Hybrid *RHCE*(2–5)	Hybrid allele		[42,43]
RHD-RHCE(4-7)-RHD	IC, TM, EF	Exon 4–7	Hybrid *RHCE*(4–7)	Hybrid allele		[42,43]
DEL *RHD*(A280T)	TM	Exon 6	838G>A (A280T)	Single missense mutation	*RHD**01EL.24 *RHD**DEL24	[42,43]
RHD-RHCE(4-9)-RHD	IC, TM, EF	Exon 4–9	Hybrid *RHCE*(4–9)	Hybrid allele	*RHD**01EL.44 *RHD**DEL44	[12]
RHD-RHCE(10)	IC, TM,	Exon 10	Hybrid *RHCE*(10)	Hybrid allele		[12]
RHD (L18P)	TM	Exon 1	53T > C(L18P)	Single missense mutation	*RHD**01EL.03 *RHD**DEL3	[12]
RHD(L84P)	TM	Exon 2	251T>C (L84P)	Single missense	*RHD**01EL.06 *RHD**DEL6	[12]
RHD(A137E)	TM	Exon 3	410C>A (A137E)	Single missense mutation	*RHD**01EL.07 *RHD**DEL7	[12]
weak D type 61	IC	Exon 1	28C>T (R10W)	Single missense mutation	*RHD**01W.61 *RHD**weak D type 61	[12]
RHD(M1I)	IC	Exon 1	3G>A (M1I)	Loss of start codon	*RHD**01EL.02 *RHD**DEL2	[12]
DEL *RHD*(X418K)	IC	Exon 10	1252T>A (X418K)	Loss of stop codon	*RHD**01EL.25 *RHD**DEL25	[32]

Rh_{null} IN CHINESE POPULATION

Rare individuals, who lack all of the known Rh antigens on their RBCs, are called Rh_{null} and most often result from consanguineous mating[1]. The Rh_{null} phenotype results in a mild clinical syndrome, called Rh-deficiency syndrome. The syndrome is characterized by chronic hemolytic anemia of varying severity, with stomatocytosis[28]. In addition, Rh_{null} RBCs have increased osmotic fragility, an altered ion transport system, and abnormal membrane phospholipids organization[29,30]. Molecular genetic studies have indicated that the Rh_{null} phenotype is transmitted in an autosomal recessive manner. Rh_{null} phenotype arises from two distinct genetic mechanisms, the regulator type, and the amorph type. The amorph type is caused by the homozygosity of a silent allele at the *RH* locus, whereas the regulator type is caused by mutations at a separate suppressor gene modulating Rh-antigen expression, which is genetically independent of the *RH* locus[31]. The Rh_{mod} phenotypes (exhibiting weak expression of Rh system antigens) are attributed to mutation of the *RHAG* gene, as are the regulator types of the Rh_{null} phenotype[32,33]. Many molecular researches have established that both *RH* and *RHAG* loci are necessary for Rh antigen expression and for function of the Rh structures as a multi-subunit complex in the RBC membrane. Most Rh_{null} cases result from different mutations in the *RHAG* gene, which has been established to be a defective regulator. It is obvious that all *RHAG* mutations reported to date are harmful and result in Rh_{null} phenotype[34]. *RHAG* gene mutation may affect the transcription and translation of the RhAG protein in several ways: missense point mutations, splice site mutations, and small exonic deletions. The *RHAG* gene was highly conserved, and all identified mutations in the *RHAG* gene were as–

sociated with the Rh_{null} phenotype. Missense mutation was the most common genetic mechanism of the Rh_{null} type. A single nucleotide change in exon 5(672C>A), causing a loss of Rh antigen expression in the Rh_{null} proband, resulting in a serine to arginine missense mutation at residue 224 was reported in Chinese[35]. Another nonsense mutation was a C to A substitution at nucleotide position 540 in exon 4, which caused a change in the amino acid tyrosine at position 180 to a stop codon. The nonsense mutation was predicted to result in a shortened RhAG protein. It was possible that the altered novel protein was not assembled in the RBC´s membrane, thus leading to Rh_{null} phenotype[36].

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Of course, blood transfusion is not the only circumstance in which blood groups need determining. Where there is blood group in compatibility between an alloimmunized mother and her fetus, the danger of HDFN, or fetal and neonatal alloimmune thrombocytopenia, means it is desirable to know the unborn baby´s blood group. Regardless of the methods used for blood type analysis, the ultimate goal is to ensure the safety of blood transfusions and prevent the occurrence of HDFN, so blood type analysis should be closely integrated with the clinical practice. DEL accounts for about 30% of the D-negative population in China. Since DEL blood from a donor is able to immunize RhD negative recipients, appropriate screening techniques such as absorption and elution tests or genetyping should be used to exclude DEL from RhD negative donors; It is same for HDFN. Also, as prenatal diagnosis of HDFN and clinical transfusion are used to treat a wide variety of individuals, all differing individual blood group characteristics must be considered. Weak D antigens have all D epitopes; partial D antigens lack one or more D epitopes. However, this is difficult to define serologically because a negative reaction with a particular monoclonal antibody or by a specific method could result from the weak expression of the epitope, rather than its absence. Individuals with partial D antigens can make anti-D; those with weak D antigens cannot. This is the usual interpretation of the dichotomy, but it is dependent on the immune response. If anti-D has not been found in any person with a particular D variant, this does not mean that another patient with the same variant will not make anti-D following immunization with RhD positive RBCs. For example, weak D type 4.2 and 15 have been classed as weak D, yet all have subsequently been found in numerous patients who have made allo-anti-D. With the development of molecular biology, new techniques such as genotyping and sequencing should be used in blood donor screening and blood transfusion departments in China. More clinical data should be accumulated for transfusion recipients and for the prenatal diagnosis of D-negative pregnant women, especially for high-prevalence *RHD* polymorphisms in China, such as *RHD**weak D type 15, *RHD**DVI.3, and DEL. With the accumulation of research and clinical data, we should be clearer as to which *RHD* variants are to be regarded as RhD negative and which need to be regarded as RhD positive.

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