Review Article

Serology and molecular biology of *DEL*: a narrative review

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Background and Objective: The Del phenotype is an antigen D positive phenotype in which the presence of antigen D can only be demonstrated by adsorption and elution of anti-D. In this narrative review, an overview of the accrued molecular and serologic knowledge is given.

Methods: Literature coverage was based on the results of an iterative PubMed recherche complemented by references from the Human RhesusBase and Rheference as well as abstracts published on Association for the Advancement of Blood and Biotherapies (AABB) and International Society of Blood Transfusion (ISBT) meetings. The time frame was from the first mention of the Del phenotype in 1984 to 2021. Non-English publications were considered if they were freely available and did not duplicate findings published in English.

Key Content and Findings: The serologic discrimination of Del from D-negative is technically difficult. The antigen density of Del is less than 50 antigens per cell. More than 45 distinct *DEL* alleles are recognized by the ISBT. The most frequent mechanisms leading to DEL alleles are missense variations and variations interfering with splicing, but several additional mechanisms including hybrid alleles, frameshift variations, premature termination codons and large deletions encompassing whole exons may underlie *DEL* alleles. The most important *DEL* allele is *RHD*01EL.01* underlying more than 90% of Del in East Asia. No cases of anti-D immunization were reported in carriers of this allele. However, anti-D immunization events were observed in carriers of other DEL alleles. In non-Asian countries, the molecular background of Del is diverse and only a tiny fraction of seemingly D negative individuals possesses a Del phenotype.

Conclusions: Up to a third of seemingly D-negative donors in East Asia carry the *DEL* allele *RHD*01EL.01*. Arguments for a D+ transfusion strategy in this allele are summarized. In other regions, only a minority of seemingly D– patients carry DEL alleles and the possible anti-D immunization of D-negative individuals by transfusion with Del blood units mistyped as D negative is the main issue. This review should help policy makers to understand the mechanisms leading to Del phenotypes, the limitations of current knowledge and the population-dependent differences in the relevance of *DEL* alleles.

Keywords: Rh blood group; *DEL*; weak D; partial D; D-negative

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Introduction: the importance of Del

The designation "Del" relates to an antigen D positive phenotype in which the presence of antigen D can only be demonstrated by adsorption and elution of anti-D while all conventional tests for antigen D including testing with anti-D in the indirect antiglobulin test (IAT) are negative (1) [in line with Association for the Advancement of Blood and Biotherapies (AABB) suggestions, in this review, Del is used for the phenotype and *DEL* for the allele].

The Del phenotype was first described by Okubo *et al.* (2) in 1984. They noticed that "some D negative red cells, though they were negative in a D^u test after exposure to anti-D, could bind anti-D and yield it on elution" (2) and called these red blood cells (RBC) D_{el}. Although their publication was officially only a "letter", most of the properties nowadays associated with the most prevalent *DEL* allele in East Asia, *RHD*01EL.01* have already been correctly identified: (I) in their (Japanese) population, about 10% of seemingly D-negative (D–) samples showed a Del

phenotype; (II) the phenotype was most often associated with the presence of antigen C: About 53% of C positive but only 3% of C negative samples were Del; (III) no Del was detected in individuals who had formed an anti-D suggesting that Del individuals were unlikely to produce an anti-D.

Almost 40 years later, the interest in *DEL* remains current because red cell genotyping enables a precision medicine approach for patients and donors with *DEL* variants. Key issues concerning Del are:

(I) In East Asian populations, a relevant fraction of seemingly D– patients and blood donors possess a Del phenotype. For example, in a large Chinese population study (3), only 1,585 of 400,253 probands (0.4%) were D– in routine serology, and 275 (18%) of them displayed a Del phenotype. In these populations, D– RBC units are scarce and their availability is affected by the transfusion strategy for Del patients: is transfusion with D-positive (D+) RBC units safe for Del patients? What is the best approach to identify Del patients?

(II) Blood donors with a Del phenotype are typed as D– with routine methods like direct agglutination by anti-D or demonstration of antigen D in the IAT. Examples of anti-D immunization caused by Del units have been reported (4). Is it necessary to screen blood donors for Del to prevent anti-D immunization by Del RBC units?

(III) Mothers with some *DEL* alleles may become anti-D immunized. How can the *DEL* allele of the mother be determined? Will her *DEL* allele allow for anti-D immunization?

(IV) Finally, in these days of whole genome or whole exome sequencing, increasingly molecular data must be analyzed without any knowledge of the serologic D phenotype. Which alleles express a Del phenotype? Is it possible to predict a Del phenotype with sufficient reliability based on molecular data alone if a previously unknown allele is detected in a proband?

Addressing these issues is crucial to define a rational transfusion strategy for patients and donors with Del phenotype. Numerous commentaries and reviews on the topic (5-11) have appeared. Recently, comprehensive reviews focusing on Del in China (10) and Japan (11) have been published. Details on specific alleles can be found in the Human RhesusBase (www.rhesusbase.info) (12) and in RHeference (www.rheference.org) (13). The International Society of Blood Transfusion (ISBT) nomenclature in this review is based on version 6.2 dated 30 September 2022 (https://www.isbtweb.org/resource/004rhd.html). As ISBT is constantly updating the tables, some alleles mentioned as "not listed" may have been included in more recent allele lists.

Here I give an overview on the serology and molecular basis of DEL with a specific focus on the remaining uncertainties in the structure-phenotype relationship and the differences between populations. This article was presented in accordance with the Narrative Review reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-22-16/rc).

Methods

A PubMed search with the terms "RHD, Del" was started (publication range 1984—the first description of the Del phenotype—to 2021; during revision a re-search was done until November 2022). "Related publications" search was done for publications related to the Del phenotype. The iterative approach was continued until no new relevant publications were identified. As second source, *DEL* alleles included in the Human RhesusBase (maintained by the author) and Rheference were analyzed for the references given. As third source, abstracts of the AABB and ISBT were screened for abstracts relevant for the Del phenotype using the term "RHD" to identify abstracts of possible relevance. For abstracts not matching previously identified publications, PubMed searches using abstract authors as key were done to identify possible related publications.

All identified publications in English defining new alleles or describing the prevalence of molecularly defined *DEL* alleles in a population were cited. Available publications giving information about immunization of Del patients or immunization by Del blood units were included if they were in English or German. Publications of mere technical merit (e.g., description of an assay for *DEL*) were not included if they did not add to the general understanding of the Del phenotype.

Non-English publications were considered if they were publicly available and contained critical information. If the same dataset was obviously published twice by the same authors in non-English and English journals, the publication in English was used as reference. If both a congress abstract and a peer-reviewed publication was identified, the peer-reviewed publication was cited.

The search strategy is summarized in *Table 1*.

Table 1 The search strategy summary

Items	Specification
Date of search	2021 (partial update 09-Nov-2022)
Databases and other sources searched	PubMed; Human RhesusBase; Rheference; ISBT *RHD* allele list; Abstracts of ISBT and AABB meetings
Search terms used	Del RHD (PubMed); RHD (abstracts)
Timeframe	1984–2021
Inclusion and exclusion criteria	All identified publications defining new *DEL* alleles, indicating a Del phenotype for a specific allele or describing the prevalence of molecularly defined *DEL* alleles in a population were included. Available publications giving information about immunization of Del patients or immunization by Del blood units were included if they were in English or German. Publications of mere technical merit (e.g., description of an assay for DEL) were not included if they did not add to the general understanding of the Del phenotype
Selection process	The selection process was performed by the single author

ISBT, International Society of Blood Transfusion; AABB, Association for the Advancement of Blood and Biotherapies.

The Asian DEL

The high frequency of the Del phenotype among seemingly D– Japanese observed by Okubo *et al.* (2) was confirmed in other Asian populations: About 30% of seemingly D– donors (0.27%) in Hong Kong possessed a Del phenotype (14), resulting in a frequency of 0.079% among all donors. In contrast, in non-Asian populations, Del represents a minuscule fraction (0.26% or less) of seemingly D– blood donors (9).

There is now overwhelming evidence that the prevalent *DEL* allele in East Asian Del probands is *RHD*01EL.01* carrying a "synonymous" c.1227G>A substitution (15-18). This allele was first dubbed *RHD(K409K)*, later *RHD(1227G>A)* and is now often referred to as "Asia type" (19) or "Asian-type" (7) *DEL*. Curiously, this allele was first identified as cause of a Del phenotype in German blood donors (20). Its "synonymous" c.1227G>A substitution in codon 409 is immediately adjacent to the exon 9/ intron 9 junction and has a detrimental effect on *RHD* splicing: most *RHD* transcripts in *RHD*01EL.01* lack *RHD* exon 9 (17,21,22).

Additional single nucleotide variations (SNVs) in intron 7 have been described for *RHD*01EL.01*-like *DEL* alleles: c.1073+152C>A (rs41307824) (15) and "c.1073+923C>T" (rs2427766) (21). The *RHD*c.[1073+152C>A, 1227G>A]* allele has been assigned a separate *DEL* allele number, *RHD*01EL.36*. A relevance of these intronic SNVs for the phenotype has been proposed (21) but never demonstrated. Most likely, these SNV do not impact the phenotype: c.1073+152C>A was detected by chance due to problems with a polymerase chain reaction (PCR) primer (15), c.1073+923T is the major allele at this SNV position among *RHD* alleles with a worldwide prevalence of 87% according to gnomAD (23).

*RHD*01EL.01* is the most frequent *DEL* allele in East Asia (11,15-17,24-29) and in most populations with a relevant admixture of individuals of East Asian descent, like Australia (30) or the USA (31). In East Asia, up to a third of individuals who type D– by routine methods carry the *RHD*01EL1.01* allele (32). Therefore, the question whether *RHD*01EL.01* patients may safely be transfused with D+ units relevantly impacts blood supply for D– individuals in these countries. An overview of surveys of anti-D immunization in *RHD*01EL.01* and other *DEL* alleles is given in *Table 2* (19,33-35). No anti-D immunization was found among 358 pregnant women and 65 transfused patients with *RHD*01EL.01*. Two studies reported in parallel data on "true" *RHD*-negative patients: Combined, there were 99 anti-D among 483 pregnant women (20%) but none among 130 pregnant women with *RHD*01EL.01* ($P<0.001$, Fisher's exact test). Likewise, there were 11 anti-D among 160 *RHD*-negative transfused patients but none among 65 transfused patients with *RHD*01EL.01* ($P<0.05$, Fisher's exact test). In conclusion, there is no evidence of anti-D alloimmunization in *RHD*01EL.01* patients in situations in which *RHD*-negative patients frequently develop an anti-D.

After several years of discussion among the experts (36), Shao (19) suggested a D+ transfusion strategy for Asian-type *DEL* based on the lack of documented anti-D immunization among *RHD*01EL.01* probands and the absence of *RHD*01EL.01* carriers among anti-D

Table 2 Surveys on anti-D immunization in *DEL* patients with *RHD*01EL.01* and with other *DEL* alleles

Population	D-negative (Del excluded)		*RHD*01EL.01*		Other *DEL* alleles		Reference
	Patients	Anti-D	Patients	Anti-D	Patients	Anti-D	
Pregnant women	155	38	44	0			(19)
Pregnant women	328	61	86	0	2	0	(33)
Transfused patients	160	11	65	0	2	0	(33)
Pregnant women (Han Chinese)	373	No data	130	0	12: *RHD*01EL.44* (n=7); *NL-8* (n=4); *RHD*01N.07* (n=1)	6: *RHD*01EL.44* (n=3); *NL-8* (n=2); *RHD*01N.07* (n=1)	(34)
Pregnant women	630	No data	98 (+70 patients excluded)	0	10: *RHD.01EL.02* (n=8); *RHD*01EL.44* (n=1); *NL-8* (n=1)	2: *RHD*01EL.44* (n=1); *NL-8* (n=1)	(35)
Patients			33	0			Clinical trial NCT03727230*

*See also https://clinicaltrials.gov/ct2/show/NCT03727230.

immunized individuals. The topic has recently been reviewed in much detail (10). D+ transfusion of patients with *RHD*01EL.01* allows to set aside D– units for those patients who really need them (10,19,35,37). The safety of D+ transfusion for *RHD*01EL.01* patients was further corroborated in a clinical trial of the Guangzhou Blood Center (NCT03727230, https://clinialtrials.gov/ct2/show/MCT0373730 accessed 26-May-2022): none of 33 Asian-type *DEL* recipients deliberately transfused with D+ blood developed an anti-D. It has even been suggested (38) that testing for *RHD*01EL.01* should be extended to patients with rare weak D phenotypes, because an *RHD*01EL.01* allele *in trans* would allow for D+ transfusion. While the logic of this policy is self-explanatory, the impact is limited: weak D and partial D probands are much rarer in China than individuals D– by routine serology [e.g., Yan *et al.* (39) observed in their donor cohort 1,401 D– donors but only 37 donors with weak D or partial D]. Furthermore, the likelihood of the presence of *RHD*01EL.01* in weak D and partial D carriers is only about half of the likelihood in a seemingly D– individual.

The difficulties to discriminate the Del phenotype from other D phenotypes

Del or partial D?

In partial D, the RhD protein is changed in a way that D epitopes are lost and immunization to normal RhD seems possible. Typical mechanisms are RhD proteins in which segments are replaced by the corresponding RhCE segments, and RhD proteins with variations in the exofacial part of the protein (1). The "partial D" phenomenon is independent of the antigen density: partial D may occur with normal or even enhanced antigen density [e.g., *RHD*03.04* (40)] as well as with reduced antigen density [e.g., *RHD*06.02* (41,42)]. If a partial D antigen occurs in a Del phenotype, a "partial Del" phenotype results. While this concept seems intuitive at first glance, the verification that a Del phenotype expresses a partial D antigen may be painstakingly difficult and sometimes impossible:

Partial D phenotypes become obvious if an allo-anti-D is produced by its carrier. Such immunization events are rare, therefore often the lack of reactivity with monoclonal anti-D that cannot be explained by a low antigen density is used as indication of a partial D phenotype. Since a partial Del phenotype does not react with an anti-D in IAT, the investigation of D epitopes has to be accomplished by adsorption/elution tests using different monoclonal anti-D (43).

The first description of a partial Del phenotype dates from 2005, when Körmöczi *et al.* (43) obtained positive elution results for *RHD*01EL.08* with 2 of 14 anti-D tested, contrasting with a uniform reactivity observed for *RHD*01EL.01*, *RHD*01EL.35*, and *RHD*11*, and an almost

Table 3 Antigen density described for Del phenotypes

Allele	Antigen density	Reference
*RHD*01EL.01*	<22	(43)
*RHD*01EL.01*	22	(46)
*RHD*01EL.08*	<22	(43)
*RHD*01EL.11*	<22	(43)
*RHD*01EL.33*	24 to 28 (median 26)	(53)
*RHD*01EL.35*	<22 to 26 (median <22)	(43)
*RHD*11*	<22 to 36 (median 33.5)	(43)
NL-5 (characterization by exon PCR)	50	(50)

PCR, polymerase chain reaction.

uniform reactivity in *RHD*01EL.25*. The identification of the absence of epitopes in partial *DEL* alleles is technically demanding. The absence or presence of reactivity with a specific antibody may depend on the method of detection used (44). It is symptomatic that *RHD*11* was among the alleles without detectable epitope loss (43) while it is considered partial D by ISBT.

Based on serologic or molecular data, a partial Del status has been assumed in at least 14 of the *DEL* alleles listed by ISBT (*RHD*01EL.04*, *RHD*01EL.05*, *RHD*01EL.08*, *RHD*01EL.09*, *RHD*01EL.19*, *RHD*01EL.22*, *RHD*01EL.23*, *RHD*01EL.31*, *RHD*01EL.33*, *RHD*01EL.42*, *RHD*01EL.44*, *RHD*01EL.47*, *RHD*01EL.48*, *RHD*01EL.49*). Two of these alleles are also listed among the partial D (*RHD01*01EL.22* as *RHD*53* and *RHD*01EL.23* as *RHD*41*).

Del or weak D?

At first glance, the discrimination of weak D and Del seems clear-cut: testing for antigen D in the indirect antiglobulin technique gives a positive result in weak D and a negative in Del. However, the result heavily depends on technical details precluding such simple distinction: Which technique has been used for the antiglobulin test? Which anti-D has been used for testing?

An antiglobulin test performed in tube technique is less sensitive than an antiglobulin test in gel (45) or even capture (46) technique. *RHD*01W.49* was known as weak D but classified as Del when tested by IAT in tube technique (3). In the same study, *RHD*01EL.06* and *RHD*01EL.07* were serologically classified as Del, but the authors observed a stronger reactivity of the eluate than in other Del and reasoned that these alleles might qualify as weak D if tested in gel technique (3). In our laboratory, the fraction of *RHD*11* samples that were missed by IAT diminished when we moved from tube technique to gel technique and on to solid phase technology.

The selection of the anti-D is critical, too. Anti-D differ in their avidity of binding to antigen D and in the D epitopes detected. In a partial Del phenotype, selection of the wrong anti-D will give a false negative result.

As additional layer of complexity, the antigen density of different samples carrying the same allele may vary. An *RHCE*02 (Ce)* allele *in trans* considerably reduces the antigen densities of weak D samples (40) and is likely to reduce the antigen density of Del samples. In addition, there is a yet unexplained "random variation" of the antigen densities of different samples with the same Rh phenotype (4,40). Even well characterized *DEL* alleles like the Asian-type *DEL RHD*01EL.01* (32,47,48) and *RHD*01EL.02* (48) are sometimes found to underlie a weak D phenotype.

Due to these confounders, the same allele may be classified as Del in one laboratory and as "weak" weak D in another. In fact, this happened for *RHD*11* that was first described as weak D (49), then observed among Del samples (20) and later was listed among the partial D. The *weak D* alleles *RHD*01W.58* (50) and *RHD*01W.61* (3,47,51) have also been reported to encode a Del phenotype. In the case of *RHD*01EL.46*, the allele is listed by ISBT both among the *DEL* alleles as *RHD*01EL.46* and among the weak D alleles as *RHD*01W.94*.

Antigen density of Del

A possibly more objective discrimination of Del and weak D might be based on the antigen density. Usually, antigen density is determined by flow cytometry (52). The measurement of D antigen density of Del samples is difficult, because there is a large overlap with the D-negative population. Weak D samples have antigen densities between 66 and 3,811 antigens/cell (40). Estimates for Del range from <22 to 22 in *RHD*01EL.01* (43,46) and up to 50 antigens/cell in other Del-like types like NL-5 (50). Overall, the data are scarce and only a few Del phenotypes have been investigated (43,46,50,53) (see *Table 3*).

Del or D-negative?

While partial D and Del are non-exclusive definitions

and the overlap between weak D and Del is explained by the variable sensitivity of antigen D testing in indirect antiglobulin technique, the difficulties to discriminate Del from D– are of technical nature:

In a partial Del, use of a monoclonal anti-D directed to D epitopes absent in the partial Del type will result in a negative adsorption/elution test and the Del status may be missed if only this anti-D is used. As often only a minority of anti-D is binding, several Del have initially been characterized as D– (e.g., *RHD*01EL.18*, *RHD*01N.07*, *RHD*01N.77*) and their Del status revealed in later studies (11,34,54,55). Likewise, old data (56) on a high frequency of samples with *RHD* intron 4 and exon 10 in Japanese D– tested negative by adsorption/elution are difficult to reconcile with current knowledge if not assuming a limited sensitivity of the adsorption/elution tests performed.

On the other hand, use of low-ionic washing buffers in the elution process may lead to false positive results due to non-specific adsorption of high-titer antibodies (57). If residual donor samples are used for characterization, contamination might also be an issue: Many blood grouping machines like the Beckman-Coulter PK series (e.g., PK 7300) machines do not use single use tips for pipetting. D+ RBCs may carry as much as 40,000 antigens per cell while Del RBCs have 30 or less. A contamination of D– RBC by 0.1% D+ would result in a positive adsorption/elution test. In conclusion, false-positive adsorption/elution is a non-trivial problem: In a recent study in Korea (58), 5.5% of *RHD* deletion samples showed a positive adsorption/elution result. These technical considerations explain why the *DEL* status of several alleles is doubtful.

Regrettably, the molecular structure is not very helpful: As detailed below, missense variations may alternatively lead to weak D, Del or D– phenotypes, the impact of splice site variations may vary, and even variations expected to abolish normal RhD expression like frameshift variations (54) or deletions of whole exons (59) may result in a Del phenotype.

Single molecule fluorescence microscopy complemented by machine learning has been suggested as alternative to adsorption/elution for the discrimination of Del from D– (44). This approach is based on fluorescence microscopy and uses the information gained from the distribution of signals on the RBC's surface to discriminate specific and non-specific binding. The suitability of the method to discriminate Del from D– was demonstrated with *RHD*01EL.08* and *RHD*09.05* samples which were also used for training of the algorithm. The method did not yet gain widespread use, possible due to the demanding technical requirements. It will be interesting to see whether it is less error-prone in routine use than adsorption/elution.

Molecular bases of Del

The first description of a correct molecular basis of a *DEL* allele was the result of a study in Germany (20) on donors harboring parts of the *RHD* gene but typed D– by direct agglutination and in IAT.

Seventeen different alleles were detected, three of which were demonstrated to encode a Del phenotype: *RHD*(M295I) (now known as *RHD*11*), *RHD*(K409K) with a c.1227G>A variation (nowadays *RHD*01EL.01*) and *RHD*(IVS3+1G>A), now known as *RHD*01EL.08*. Hence, missense variations and splice site variations were recognized as major molecular mechanisms leading to Del phenotypes already in this study.

Including the "Asian-type" *DEL* allele *RHD*01EL.01*, the current ISBT *RHD* allele table version 6.2 dated 30 September2022 lists 48 *DEL* alleles (*Table 4*) (3,4,15,20,27,30,51,53-55,59,63,64,66-69,71,76,79,81, 83-85,87). In three of these alleles (*RHD*01EL.32*, *RHD*01EL.35*, *RHD*01EL.37*), the polymorphism indicated in the ISBT table as difference to normal *RHD* is unlikely to cause the Del phenotype, because the variations are frequent or even present (*RHD*01EL.37*) in the NCBI reference sequence NG_007494.1.

Ten additional *RHD* alleles have been mentioned to be associated with a Del phenotype but are not acknowledged by ISBT as *DEL* and listed as partial D (*RHD*09.05*, *RHD*11*), weak D (*RHD*01W.58*, *RHD*01W.61*) or D– (*RHD*01N.07*, *RHD*01N.48*, *RHD*01N.60*, *RHD*01N.67*, *RHD*01N77*). The other way around, in five *DEL* alleles acknowledged by ISBT (*RHD*01EL.13*, *RHD*01EL.15*, *RHD*01EL.20*, *RHD*01EL.28*, *RHD*01EL.39*) the evidence for a Del phenotype is weak, because peer-reviewed publications or independent descriptions in abstract form are lacking. Finally, ten structures (*NL-1* to *NL-10)* have been reported to be associated with a Del phenotype but have not been included in the ISBT allele tables yet.

About two thirds of *DEL* alleles harbor missense variations in *RHD* (15 *DEL* alleles listed by ISBT plus 6 other) or splice site variations (14 listed by ISBT plus 3 other). The remaining third (18 listed by ISBT plus 10 other) are caused by many different mechanisms: Rearrangements of the *RH* locus including *RHD/RHCE* hybrid alleles (2 listed by ISBT plus 4 other), large deletions encompassing at least one *RHD* exon, variations

Table 4 Molecular bases of *DEL*

ISBT name	Nucleotide variations[$]	Predicted protein variations[$]	Haplotype/phenotypes[$]	Mechanism	Comments	Reference
*RHD*01EL.01*	c.1227G>A	p.=	DCe	Splice site variation (exon 9/IVS 9)[‡]	Asian-type *DEL*, prevalent in East Asia	(20)
*RHD*01EL.02*	c.3G>A	p.Met1?	Cce and cEe (3)	Loss of start codon		(51)
*RHD*01EL.03*	c.53T>C	p.Leu18Pro	Cce (3)	Single missense variation		(51)
*RHD*01EL.04*	c.[147del, 148+6del]		DCe	Deletion of A at position 147 causing frameshift	AA>A; the deletion of c.148+6A is not mentioned by ISBT but seemingly always present when investigated (54). Anti-D immunization reported (60)	(54)
*RHD*01EL.05*	c.148+1G>A		DcE	Splice site variation (exon 1/IVS 1)[§§]	Mentioned in abstract form in 2001 (61)[†]	(30)
*RHD*01EL.06*	c.251T>C	p.Leu84Pro	cEe (3)	Single missense variation	Antigen density higher than Asian-type *DEL*, weak D excluded in tube technique only (3)	(51)
*RHD*01EL.07*	c.410C>A	p.Ala137Glu	Cce (3)	Single missense variation	Antigen density higher than Asian-type *DEL*, weak D excluded in tube technique only (3)	(51)
*RHD*01EL.08*	c.486+1G>A		DCe	Splice site variation (exon 3/IVS 3)[§]	Partial Del phenotype (43). 19 amino acid insertion after Asn162 predicted (62)	(20)
*RHD*01EL.09*	c. 486+2T>A		DcE and DCe	Splice site variation (exon 3/IVS 3)[§]	Del phenotype observed in CcDee (30)	(54)
*RHD*01EL.10*	c.1222T>C	p.Trp408Arg	DCe	Single missense variation		(27)
*RHD*01EL.11*	c. 1252dup	p.Ter418LeuextTer72	DCe	Duplication of c.1252T with loss of stop codon	TTTT>TTTTT	(63)
*RHD*01EL.12*	c.458T>C	p.Leu153Pro	cEe	Single missense variation		(54)
*RHD*01EL.13*	c.786del		DCe	Deletion of c.786A causing frameshift	AA>A; reported as D– (54)	(54)
*RHD*01EL.14*	c.634+5G>T		DCe	Splice site variation (exon 4/IVS 4)[‡]	Reported as weak D (64)	(64)
*RHD*01EL.15* *RHD*01N.52*	c.922G>T	p.Gly308Ter	DCe	Nonsense variation	Reported as Del in abstract form (65)[†] probably due to false-positive adsorption/elution; most likely D– (66)	(66)
*RHD*01EL.16*	c.634G>C	p.Gly212Arg	Dce	Missense (splice site exon 4/IVS 4 affected)[‡]		(54)
*RHD*01EL.17; RHD*01N.22*	c.1203T>A	p.Tyr401Ter	DcE	Nonsense variation	Initially described as D– by routine serology. Del phenotype first reported by Flegel *et al.* (54)	(63)
*RHD*01EL.18; RHD*01N.50*	c.93dup	p.Thr32TyrfsTer4	DCe	Duplication of c.93T causing frameshift	TTTTTTT>TTTTTTTT; Del phenotype first reported by Flegel *et al.* (54)	(67)
*RHD*01EL.19*	c.635-2A>G		Not reported	Splice site variation (IVS 4/exon 5)[§]	Del phenotype mentioned in HE577129	(68)
*RHD*01EL.20*	c.1154-8T>A		Not reported	Splice site variation (IVS 8/exon 9)[‡]	No detailed serology available	(69)
*RHD*01EL.21*	c.148+5G>C		Cce	Splice site variation (exon 1/IVS 1)[#]		(30)
*RHD*01EL.22; RHD*53*	c.336-2del		Cce	Deletion of 336-2A causing splice site variation (exon 2/IVS 2)[§]	Partial Del phenotype according to genbank entry KC341996	(30)
*RHD*01EL.23; RHD*DBU; RHD*41*	*RHD-cE*(5-7)-D	RhD-cE(5-7)-D	DcE	hybrid allele	Substitution of *RHD* exons 5 to 7 by the corresponding exon 5 to 7 of the cE allele of *RHCE*; partial Del phenotype expected	(54)
*RHD*01EL.24*	c.838G>A	p.Ala280Thr	Cce	Single missense variation	No impact on exon 6 splicing in minigene splicing assay (70)	(55)
*RHD*01EL.25*	c.1252T>A	p.Ter418LysextTer26	DCe	Loss of stop codon		(26)
*RHD*01EL.26*	c.1247dup	p.Phe417IlefsTer73	DCe	Duplication of 1247G causing frameshift	The allele is designated *RHD*1248G* in two manuscripts (71,72). The accompanying genbank entry KJ145906.1 displays an insertion before position 1248 (c.1247_1248 insG or c.1247dup). However, the ISBT table lists an insertion after position 1248 (c.1248_1249insG) which would result in p.Phe417ValfsTer73	(71)

Table 4 (*continued*)

Table 4 (*continued*)

ISBT name	Nucleotide variations[$]	Predicted protein variations[$]	Haplotype/phenotypes[$]	Mechanism	Comments	Reference
*(RHD*01EL.27)*	Not used*					
*RHD*01EL.28*	c.993del	p.Phe332SerfsTer28	not reported	Deletion c.993C causing frameshift	No phenotype reported in LN680540	†
*RHD*01EL.29*	c.1210G>C	p.Asp404His	DcE	Single missense variation	Reported as Del in JX114749	†
*RHD*01EL.30*	c.1074-649_1153+266del	p.359_384delins56[$]	Not reported	Large deletion encompassing exon 8	In the longest transcript, exon 8 (80 nucleotides) is replaced by an intron 7 segment comprising 170 nucleotides	(59)
*RHD*01EL.31*	c.148+1G>T	IVS1+1G>T	DcE	Splice site variation (exon 1/IVS 1)[‡‡]	Partial Del (73)	(71)
*RHD*01EL.32*	c.149-29G>C		DCe	Intron polymorphism	This polymorphism is frequent in D+ alleles and not causative of the phenotype (74). According to gnomAD (23), the frequency of c.149-29G>C is 0.600. c.149-29G>C is typical for *RHD* alleles in Dce, DCe or DCE haplotypes (75)	(76)
*RHD*01EL.33*	c.336-2A>G		DCe	Splice site variation (IVS 2/exon 3)[$]		(53)
*(RHD*01EL.34)*	Not used*					
*RHD*01EL.35*	c.802-41_802-38del; c.802-38_35del (ISBT)		DCe	Intron polymorphism	An allele with a c.802-41_802-38 del was described in 2005 (4) and listed by ISBT as *RHD*01EL.35* for many years. The polymorphism in IVS5 is frequent (77) and may not be the cause of the Del phenotype. According to gnomAD (23), the frequency of c.802-41_802-38del is 0.071. Since version 6.0 of the *RHD* allele tables, a different position of the deletion is indicated but no source given	(4)
*RHD*01EL.36*	c.[1073+152C>A,1227G>A]		DCe	Splice site variation (exon 9/IVS 9)	The polymorphism in IVS7 was detected because it interfered with primer binding; an influence on the phenotype is not documented	(15)
*RHD*01EL.37*	c.1154-31C>T		Not reported	Intron polymorphism	Mentioned in abstract form (65). The polymorphism in IVS8 is frequent and may not cause the phenotype. The T is present in the NCBI reference sequence NG_007494.1. According to gnomAD (23), the frequency of c.1154-31T is 0.775. c.1154-31C is typical for *RHD* alleles in a DcE haplotype (75)	†
*RHD*01EL.38; RHD*01N.57*	c.1010T>G	p.Leu337Arg	DCe	Single missense variation	Adsorption/elution not tested in original publication. Slightly reduced exon inclusion of exon 6 (80%) in minigene splicing assay (70)	(71)
*RHD*01EL.39*	c.113T>A	p.Leu38Ter	Not reported	Nonsense variation	Reported as Del in abstract form (65)[†] probably due to false-positive adsorption/elution (78)[†]	†
*RHD*01EL.40*	c.278T>G	p.Leu93Arg	Cce	Single missense variation		(71)
*RHD*01EL.41*	c.872C>G	p.Pro291Arg	Cce	Single missense variation	Slightly reduced exon inclusion of exon 6 (80%) in minigene splicing assay (70)	(79)
*RHD*01EL.42*	c.[149-29G>C,335G>C]	p.Ser112Thr	Not reported	Missense (splice site affected exon 2/IVS 2)[§]		(79)
*RHD*01EL.43*	c.46T>C	p.Trp16Arg	DcE	Single missense variation	Also reported as weakly positive in IAT (79)	(79,80)
*RHD*01EL.44*	*RHD-RHCE*(4-9)-RHD	RhD-RhCE(4-9)-RhD	DCe	Hybrid allele	Partial Del (34)	(3)
*RHD*01EL.45*	c.721A>C	p.Thr241Pro	Not reported	Single missense variation		(66)
*RHD*01EL.46 RHD*01W.94*	c.884T>C	p.Met295Thr	DCe or DcE	Single missense variation	Initially described as weak D (81,82); later reported as Del (66)	(81)
*RHD*01EL.47*	c.510dup	p.His171AlafsTer28	cEe	Frameshift (duplication of 510G)	Partial Del (83)	(83)
*RHD*01EL.48*	c.1154-412_1227+526del		Not reported	Large deletion encompassing exon 9	Also designated DKG; partial Del (weakly positive with 4 of 12 anti-D tested)	(84)
*RHD*01EL.49*	c.1016G>T	p.Gly339Val	Cce	Single missense variation	Probably partial Del (85); loss of epD6.6, 8.2 and 9.1	(85)
*RHD*01EL.50*	c.1151C>G	p.Thr384Arg	Cce	Missense variation	First observed with “unknown phenotype” (86); Del phenotype shown in (87)	(87)

Table 4 (*continued*)

Table 4 (*continued*)

ISBT name	Nucleotide variations[$]	Predicted protein variations[$]	Haplotype/phenotypes[$]	Mechanism	Comments	Reference
*RHD*09.05*	c.[602C>G,667T>G,819G>A,872C>G]	p.[Thr201R,Phe223Val,Pro291Arg]	Dce	Multiple missense variations	Known as weak D type 4.3; structurally closely related to weak D type 4.0 (*RHD*09.03.01*). Phenotype is Del, no data on partial D phenotype	(88)
*RHD*11*	c.885G>T	p.MetM295Ile	DCe	Single missense variation	Initially reported as weak D in a Dce haplotype (49). More frequent in DCe, usually with a borderline weak D/Del phenotype; allo-anti-D described. Slightly reduced exon inclusion of exon 6 (70%) in minigene splicing assay (70)	(20)
*RHD*01N.07*	*RHD-RHCE(4-7)-RHD*	RhD-RhCE(4-7)-RhD	Cce	Hybrid allele	Initially reported as D– in DcE haplotype (89); later found in Chinese C+c+E-e+ Del sample (55). Partial Del (34)	(89)
*RHD*01N.48*	c.822delG	p.Leu275TrpfsTer13	Cce	Frameshift	Described as D– in genbank entry HG779212 but listed as adsorption/elution positive in the accompanying manuscript (79). Deletion is at position c.822, not c.882	(79)
*RHD*01N.60*	c.1213C >T	p.Gln405Ter	CcEe	Nonsense variation exon 9	Eluate weakly positive (11)	(11)
*RHD*01N.67*	c.1-15144_148+3158del		Dce	Large deletion exon 1	Deletion characterized in Del sample (90)	(90)
*RHD*01N.77*	c.1228-1G>A		Cce	Splice site variation (IVS 9/exon 10)[§§]	Eluate 2+ (11)	(71)
*RHD*01W.58*	c.1006G>C	p.Gly336Arg	Cce	Single missense variation	Very weak D, may appear as Del (50). Almost normal exon inclusion of exon 6 (>80%) in minigene splicing assay (70)	(69)
*RHD*01W.61*	c.28C>T	p.Arg10Trp	DCe	Single missense variation	Generally known as weak D [AM412754, (82,91)]; reported as Del in China (3,47,51)	(51)
Not listed; NL-1	c.1154-374_1227+563del		Not reported	Large deletion encompassing exon 9	This 1,013 bp deletion is different from the deletion in *RHD*01EL.58* and was initially described to underlie most Asian Del samples (92). If the allele exists it must be rare.	(92)
Not listed; NL-2	c.486+5G>A		cEe	Splice site variation (exon 3/IVS 3)[§]	No serology in initial publication (69); characterized both as Del (79) and as weak D (93)	(69)
Not listed; NL-3	RHCE(1-8)-D(9-10)		DCe	Structure not fully characterized	*RHD* exons 1 to 7 are present but transcript analysis showed truncated *RHD(1-7)*; *RHCE(1-8)-RHD(9-10)* and normal RHCE transcripts (94)	(94)
Not listed; NL-4	c.[602C>G, 667T>G, 819G>A, 919G>A]	p.[Thr201Arg,Phe223Val,Gly307Arg]	Not reported	Multiple missense variations	Characterized as Del in (66). Reduced exon inclusion of exon 6 (50%) in minigene splicing assay (70)	(95)
Not listed; NL-5	c.1227+2874_1254+1317del		DCe	Large deletion exon 10 (RHD ex10 del type 1)	Initially described as weak D (96). Also observed in Del samples (79,93,97). The exact position of the deletion is not described in all reports relating to Del phenotype (79,93)	(96)
Not listed; NL-6	*RHD(1-9)-RHCE(10)*	p.=	DCe	Hybrid allele	Hybrid structure supported by cDNA analysis (76). As the protein structure is identical to RhD, the cause of the Del phenotype is unknown	(76)
Not listed; NL-7	c.93T>A	p.Phe31Leu	Cce	Single missense variation		(55)
Not listed; NL-8	*RHD-RHCE(2-5)-RHD*	RhD-RhCE(2-5)-RhD	Not reported	Hybrid allele	Differs from DVI type IV (98) by a definitive *RHCE* origin of exon 2. Partial Del (34)	(55)
Not listed; NL-9	c.487-1G>A		Cce	Splice site variation (IVS 4/exon 4)		(86)
Not listed; NL-10	c.1027del	p.Tyr343ThrfsTer16	cEe	Frameshift		(99)

[$]The variations are described according to the HGVS recommendations for sequence variant nomenclature (100). The haplotypes (Dce, DcE, DCe or DCE) are indicated as reported in the reference or deduced from independent reports. If only a single sample or discordant samples were reported, the antigens C, c, E and e present in the samples are indicated [e.g., cEe if the sample was C-c+E+e+]. **RHD*01EL.27* and *RHD*01EL.34* have never been used in an official ISBT *DEL allele* listing. [#]No impact on splicing in minigene splicing assay (101). [‡,‡‡]Full length exon inclusion maintained in minigene splicing assay; references: [‡](102), [‡‡](101). [§,§§]No full length exon inclusion transcripts observed in mini-gene splicing assay (probable partial Del); references [§](102), [§§](101). [†]Described in abstract form or as genbank entry only. ISBT, International Society of Blood Transfusion; IVS, intervening sequence (Intron); IAT, indirect antiglobulin test.

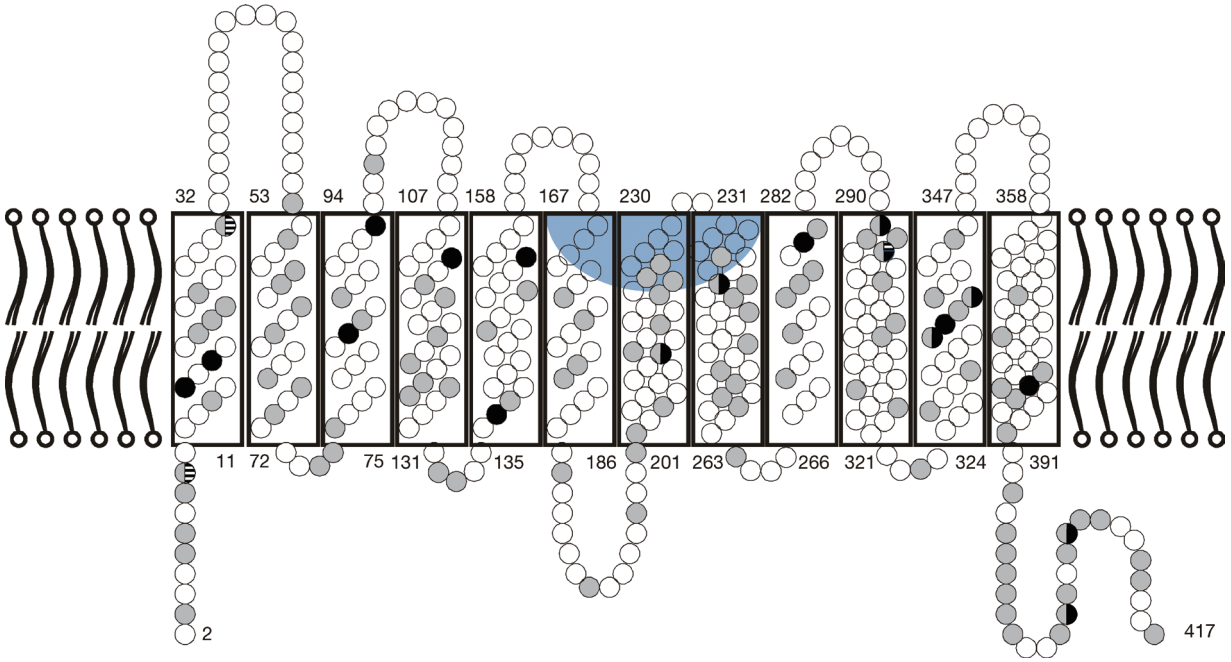

Figure 1 Amino acid positions involved in Del and weak D phenotypes. A schematic model of the RhD protein in the membrane (103) is shown. The amino acid positions are shown as disks. The RhD protein forms 12 transmembrane helices, six exofacial loops, five intracellular loops, and an intracellular N-terminal and C-terminal segment. Near exofacial loop 4, amino acids located within the membrane are accessible for antibodies (dark gray region). The codon position of single missense variations involved weak D phenotypes (according to ISBT) is indicated by gray disks, those involved in Del phenotypes by black disks. Disks with stripes indicate single missense variations in Del phenotypes not listed as *DEL* by ISBT. Both in Del and in weak D, the underlying amino acid substitutions are generally located in the transmembrane and intracellular regions of the RhD protein. ISBT, international society of blood transfusion.

in the termination codon leading to an elongated protein, variations in the start codon, duplications or small deletions introducing a frameshift and nonsense variations introducing a premature stop codon. The listing of molecular causes is certainly incomplete: the alterations described in three alleles cannot be the cause of a Del phenotype and there have been repeated observations (26,54,66,99) of a Del phenotype in samples with a seemingly normal *RHD* gene indicating that some causes of *DEL* cannot be found by currently used methods.

DEL alleles with missense variations

There are 21 alleles expressing a Del phenotype that carry a single missense variation. Two additional alleles carry a missense variation on a weak D type 4.0 (*RHD*09.03.01*) background. Similar to *weak D* alleles (49), the missense variations are located in the intracellular or transmembrane segments of the RhD protein [*Figure 1* (103)].

Missense variations may lead to a reduced antigen density by two non-exclusive mechanisms (listed in the order of interference with protein synthesis):

(I) Sometimes, the altered nucleotide sequence may interfere with correct splicing. In two of the 23 alleles (*RHD*01EL.16* and *RHD*01EL.42*), the missense variation is adjacent to an exon/intron junction and the Del phenotype most likely caused by an effect on splicing (102). However, even SNV in the exon not directly adjacent to an intron/exon junction may cause incorrect splicing (70,104).

(II) Missense variations impact the protein structure and may disrupt protein folding, intramolecular interactions, and intermolecular interactions with partners leading to reduced protein integration into the membrane or antigen loss. When the effect of the 23 Del-associated missense variations on protein structure is evaluated using prediction tools like PROVEAN (105), SIFT (106), or PolyPhen-2 (107),

all missense variations are predicted to be damaging by at least one tool and 16 are considered damaging by all tools (*Table 5*). A similar result is obtained for weak D types associated with single missense variations (the 23 *DEL* alleles were compared with the same number of weak D alleles, which covers the range weak D type 1 to type 27, because type 4, type 11 and type 15 are not included in the ISBT weak D list and type 14 is not due to a single SNV): 2 alleles carry missense variations likely to affect splicing; all but two alleles carry variations considered deleterious by at least one tool and 15 are considered damaging by all tools. If different variations in the same codon cause either weak D or Del phenotypes, the variation causing the Del phenotype usually has a more detrimental PROVEAN score than those causing weak D or borderline weak D/Del phenotypes (e.g., *RHD*01EL.10*: –10.563 *vs. RHD*01W.22*: –9.747). However, the variation of scores observed for different codons is so large that current prediction tools must be considered insufficient to discriminate missense variations causing Del from those causing weak D phenotypes.

DEL alleles with splice site variations

The variations in many Del including the Asian-type *DEL RHD*01EL.01* interfere with splicing. Usually, these variations are near the exon/intron boundary, most often in the intron. In seven alleles (ISBT: 6), the variation is 3' of the exon within 6 bp from the exon/intron junction, in six alleles (ISBT: 4), it is 5' of the exon, and in four alleles (all listed by ISBT), it is within the exon. This list may be too short as variations in the exon not adjacent to the exon/intron junction (104) or deep in the intron (108) may impact splicing.

Splice site variations may diminish or completely abolish the production of normal *RHD* transcripts. Incorrect splicing often results in the incorporation of intron components as pseudoexons: in RhD*01EL.08, a 19 amino acid insertion after Asn162 is predicted (62); *RHD*01EL.01* transcripts often contain parts of intron 7 (21). If normal *RHD* transcripts are retained, normal RhD is present in the membrane and anti-D immunization of the carrier of these alleles is unlikely (102). In contrast, variations causing total absence of normal transcripts like c.486+1G>A in *RHD*01EL.08* are likely to allow for anti-D immunization and to express a partial Del phenotype.

The most important example of a *DEL* allele with disrupted splicing is the Asian type *DEL* allele *RHD*01EL.01*. In this allele, the SNV bordering the intron/exon junction leads to exclusion of exon 9. Analysis of mRNA indicated that no transcripts with exon 9 were maintained (109). Transcripts lacking *RHD* exon 9 encode for a 463 amino acid protein that differs from standard RhD starting at position 385. The differences of this altered protein to the standard RhD protein are almost exclusively located in the C-terminal intracellular part of the protein. Therefore, such altered RhD protein might be able to integrate into the membrane resulting in a Del phenotype despite the lack of the ankyrin binding site. This interpretation is supported by the Del or weak D phenotype of some alleles with genomic deletions of *RHD* exon 9 (84) or 10 (96). However, the *RHD* exon 9 deletion allele leads to a partial D phenotype (84) while no anti-D observed was observed in *RHD*01EL.01* probands (46). Therefore, the most likely explanation is the presence of a tiny number of normal transcripts missed by mRNA analysis. This interpretation is supported by a minigene splicing assay (102) in which full-length transcripts including *RHD* exon 9 were maintained in *RHD*01EL.01*.

Generally, minigene splicing assays (101,102) have the advantage that they allow for a systematic analysis. Still, there are limitations: the results obtained may depend on the assay used (110). Some intronic variations within the splice consensus sequence like c.148+5G>C in *RHD*01EL.21* reportedly cause a Del phenotype but have limited impact in splicing assays (101). In *RHD*01EL.31*, the presence of normally spliced transcripts was predicted (101) but the phenotype is a partial Del (73). In conclusion, while analysis of mRNA and minigene splicing assays help to understand the impact of splice-site variations, they cannot replace serology yet. It is important that serological data are solid and confirmed by independent observations, especially if they are discrepant to the phenotype expected based on molecular studies.

DEL alleles with hybrid structure

Six alleles with hybrid structure have been correlated with a Del phenotype. Only two of these alleles (*RHD*01EL.23* and *RHD*01EL.44*) are listed as *DEL* in the current ISBT table.

Typically, RhCE-like segments in RhD lead to the loss of distinct RhD epitopes (41,111). Alleles with substitutions

Table 5 Predicted impact of missense variations observed in Del and weak D alleles

Phenotype	Allele	Protein variations	Provean (105)		SIFT (106)		PolyPhen-2 (107)	
			Score	Prediction	Score	Prediction	Probability	Prediction
Del	*RHD*01EL.03*	p.Leu18Pro	–5.565	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.06*	p.Leu84Pro	–4.587	Deleterious	0.15	Tolerated	1	Probably damaging
	*RHD*01EL.07*	p.Ala137Glu	–2.031	Neutral	0.01	Deleterious	0.326	Benign
	*RHD*01EL.10*	p.Trp408Arg	–10.563	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.12*	p.Leu153Pro	–2.036	Neutral	0.04	Deleterious	0.4	Benign
	*RHD*01EL.16*	p.Gly212Arg†	–7.327	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.24*	p.Ala280Thr	–3.311	Deleterious	0.01	Deleterious	0.976	Probably damaging
	*RHD*01EL.29*	p.Asp404His	–5.404	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.38*	p.Leu337Arg	–4.624	Deleterious	0.01	Deleterious	1	Probably damaging
	*RHD*01EL.40*	p.Leu93Arg	–5.039	Deleterious	0.00	Deleterious	0.998	Probably damaging
	*RHD*01EL.41*	p.Pro291Arg	–7.991	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.42*	p.Ser112Thr†	–2.091	Neutral	0.01	Deleterious	0.993	Possibly damaging
	*RHD*01EL.43*	p.Trp16Arg	–5.567	Deleterious	0.18	Tolerated	0.71	Possibly damaging
	*RHD*01EL.45*	p.Thr241Pro	–5.544	Deleterious	0.00	Deleterious	0.999	Probably damaging
	*RHD*01EL.46*	p.Met295Thr	–5.422	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.49*	p.Gly339Val	–7.073	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01EL.50*	p.Thr384Arg	–4.606	Deleterious	0.07	Tolerated	1	Probably damaging
Del-like	*RHD*09. 05*	p.[Thr201R, Phe223Val, Pro291Arg]	–7.991	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*11*	p.MetM295Ile	–3.622	Deleterious	0.02	Deleterious	1	Probably damaging
	*RHD*01W.58*	p.Gly336Arg	–5.245	Deleterious	0.00	Deleterious	0.985	Probably damaging
	*RHD*01W.61*	p.Arg10Trp	–6.475	Deleterious	0.00	Deleterious	1	Probably damaging
	NL-4	p.[Thr201Arg,Phe223 Val,Gly307Arg]	–3.121	Deleterious	0.03	Deleterious	0.993	Probably damaging
	NL-7	p.Phe31Leu	–4.163	Deleterious	0.10	Tolerated	0.105	Benign
Weak D	*RHD*01W.01*	p.Val270Gly	–6.300	Deleterious	0.00	Deleterious	0.248	Benign
	*RHD*01W.02*	p.Gly385Ala†	–4.861	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.03*	p.Ser3Cys	–3.447	Deleterious	0.01	Deleterious	1	Probably damaging
	*RHD*01W.05*	p.Ala149Asp	–4.267	Deleterious	0.00	Deleterious	0.981	Probably damaging
	*RHD*01W.06*	p.Arg10Gln	–3.322	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.07*	p.Gly339Glu	–6.146	Deleterious	0.00	Deleterious	0.878	Possibly damaging
	*RHD*01W.08*	p.Gly307Arg	–3.121	Deleterious	0.03	Deleterious	0.993	Probably damaging
	*RHD*01W.09*	p.Ala294Pro	–4.053	Deleterious	0.01	Deleterious	1	Probably damaging
	*RHD*01W.10*	p.Trp393Arg	–9.563	Deleterious	0.00	Deleterious	1	Probably damaging

Table 5 (*continued*)

Table 5 (*continued*)

Phenotype	Allele	Protein variations	Provean (105)		SIFT (106)		PolyPhen-2 (107)	
			Score	Prediction	Score	Prediction	Probability	Prediction
	*RHD*01W.12*	p.Gly277Glu	–7.327	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.13*	p.Ala276Pro	–4.569	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.16*	p.Trp220Arg	–12.962	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.17*	p.Arg114Trp	–2.610	Deleterious	0.02	Deleterious	0.999	Probably damaging
	*RHD*01W.18*	p.Arg7Trp	–3.236	Deleterious	0.00	Deleterious	0.034	Benign
	*RHD*01W.19*	p.Ile204Thr	1.547	Neutral	0.51	Tolerated	0.008	Benign
	*RHD*01W.20*	p.Phe417Ser	–2.753	Deleterious	0.00	Few data	0.999	Probably damaging
	*RHD*01W.21*	p.Pro313Leu	–3.836	Deleterious	0.05	Tolerated	0.287	Benign
	*RHD*01W.22*	p.Trp408Cys	–9.747	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.23*	p.Gly212Cys[†]	–8.344	Deleterious	0.00	Deleterious	1	Probably damaging
	*RHD*01W.24*	p.Leu338Pro	–4.381	Deleterious	0.01	Deleterious	1	Probably damaging
	*RHD*01W.25*	p.Arg114Gln	–0.003	Neutral	0.44	Tolerated	0.083	Benign
	*RHD*01W.26*	p.Val9Asp	–4.700	Deleterious	0.00	Deleterious	0.087	Benign
	*RHD*01W.27*	p.Pro221Ser	–7.406	Deleterious	0.00	Deleterious	1	Probably damaging

[†]Variation probably affects splice site (102).

encompassing exon 3 to exon 7 have initially been described as D– (20), those with smaller substituted segments express a distinct partial D phenotype (41,111). Often, the D antigen density of hybrid alleles is considerably reduced even if using anti-D binding to epitopes retained in these hybrids. For example, the antigen density of *RHD*06.01* is about 402 antigens compared to 19,000 in a DcEe control (42). There is no direct relationship between the extent of the substitution and the antigen density (42).

Based on their structure, *DEL* alleles with hybrid structure are expected to express a partial Del phenotype. The lack of distinct D epitopes may lead to a misclassification as D–: use of anti-D binding to D epitopes absent in the phenotype will result in a negative adsorption/elution test. Often, only a minority of anti-Ds are binding, like 4 of 16 in *RHD*01EL.44* (34). It is therefore not surprising that some alleles like *RHD-RHCE(4-7)-RHD* (*RHD*01N.07*) were initially considered D– but later repeatedly reported to underlie Del samples (34,55).

The situation is further complicated by the usually incomplete characterization of hybrid alleles: Several alleles were characterized only regarding the presence and absence of *RHD* exons, disregarding breakpoints or exon sequences. Therefore, it is difficult to discern if different phenotypes observed for the "same" allele in different laboratories are due to differences in the methods used for serologic characterization or due to the investigation of probands carrying different alleles.

DEL alleles with frameshift variations

At first glance, the expression of antigen D by an *RHD* allele with a frameshift variation is surprising. These variations are expected to lead to RhD proteins in which large protein segments do not share homology with RhD. The impact of such frameshift variations and possible mechanisms for residual D expression have recently been analyzed by Flegel and Srivastava (112): expression of D antigen was observed in 8 of 51 alleles with a frameshift variation. In five of these alleles, including four Del alleles, there were convincing explanations for the residual D expression, like the possible use of alternate start codons in alleles with variations in exon 1 (*RHD*01EL.04* and *RHD*01EL.18*), transcriptional or translational frameshifting (*RHD*01EL.18*) or very limited changes in the C-terminal end of the protein with extension (*RHD*01EL.11* and *RHD*01EL.26*). For other

alleles, the Del phenotype was sometimes difficult to explain. For example, in *RHD*01EL.47* a duplication of G at position 510 leads to a predicted protein of only 199 amino acids (compared to 418 in RhD) that shares only the first 171 amino acids with RhD. Still, a partial Del phenotype was reported, in which 3 of 21 anti-D were positive in the adsorption/elution test (83). For some of the remaining *DEL* alleles with frameshift, the Del phenotypes have never been thoroughly evaluated by serology and a D– phenotype with a false-positive adsorption/elution result cannot be excluded.

DEL alleles with premature termination codons

There are several reports of a Del phenotype expressed by alleles with a termination codon causing a truncation of the carboxy terminal intracellular end of the RhD protein [codon 401 in *RHD*01EL.17* (54), codon 405 in *RHD*01N.60* (11)]. Possibly, loss of the carboxy terminal part of RhD does not fully abolish antigen D expression. In contrast, the expression of antigen D by RhD proteins prematurely terminated in the middle (*RHD*01EL.15* with termination at codon 308) or start (*RHD*01EL.39* with termination in codon 38) of the RhD protein is more puzzling. False-positive results in adsorption/elution testing are a likely explanation for these alleles.

DEL alleles with major alterations in the RH gene including deletions of whole exons or loss of start or stop codon

RhD is part of a trimeric complex with RhAG (113), and minor alterations like missense variations in RhAG and or RhD may severely interfere with antigen D expression. Therefore, it is surprising that major alterations of the *RHD* gene like deletions of whole exons or loss of the normal start codon may still allow for D expression resulting in a Del phenotype.

Apart from the deletion of exon 6, each deletion of an *RHD* exon leads to a loss of the reading frame. However, missing exons might be replaced by pseudo-exons derived from introns. For example, in *RHD*01EL.30* the 80 nucleotides of exon 8 are replaced by a 170 bp segment from intron 7 (59) retaining the reading frame for exons 9 and 10. Hence the true impact on protein structure may be less than expected.

So far, whole exon deletions in *DEL* alleles have been restricted to exon 1, 8, 9, and 10. Possibly, the protein segments encoded by these DNA segments are not essential for the formation of the Rh complex.

In case of *RHD* exon 1, there are several data hinting in this direction: (I) *RHD* alleles with variations in the start codon may express a Del phenotype. The most well-known example is *RHD*01EL.02*, the second most frequent *DEL* allele in many Asian populations; (II) *RHD* alleles with deletions of exon 1 may express a Del phenotype; (III) the bacterial RH homologue AmtB lacks the first transmembrane segment yet forms a Rh complex-like trimeric structure (114).

RHD exons 8 to 10 encode protein segments that form the last transmembrane protein segment and the intracellular tail of the RhD protein. Obviously, these segments have limited importance for the Rh complex: *RHD* alleles with deletions of exon 8, exon 9 or exon 10 may express RhD antigen, variations in the termination codon leading to elongated proteins often also result in a Del phenotype.

DEL alleles with exon deletions usually express a partial Del phenotype. The deletions may be triggered by homologous regions surrounding the deleted sequence: In *RHD*01EL.48* (DKG), there is a 25 bp sequence identical in IVS 8 and IVS 9 (84).

Another major alteration of the RH genes is observed in *NL-3*: In this allele, *RHCE* exons 1 to 8 seem to be linked to *RHD* exon 9 and 10 (76), but *RHD* exons 1 to 7 are present yet seemingly non-expressed. The origin of the D antigen in this haplotype is unknown: RhD(1–7) seems to be unexpressed. RhCE-D(9 to 10) differs from RhCE by a single amino acid located in the C-terminal intracellular protein segment and it is difficult to imagine how such change could lead to antigen D expression.

DEL alleles with "normal" coding sequence

Repeatedly, Del phenotypes were observed in samples for which no alteration in the *RHD* allele could be demonstrated (26,54,66,115). In addition, three *DEL* alleles (*RHD*01EL.32*, *RHD*01EL.35*, and *RHD*01EL.37*) have initially been characterized by the presence of alterations in an intron (c.149-29G>C, c.802-41_802-38del, and c.1154-31C>T) that later turned out to represent frequent intron polymorphisms (74,77) also present in *RHD* alleles with normal RhD expression. Both observations suggest that the current list of possible causes of a Del phenotype is incomplete.

Worldwide distribution of Del

The distribution of *DEL* alleles is considerably different between East Asian and European populations (*Table 6*) (3,4,26,27,29-31,62,63,66,72,76,79,93,94,99,116,119,121, 129,133,137,139) implicating a different importance of the Del phenotype for transfusion strategies:

In typical East Asian populations, donors D– by routine serology are rare and usually comprise less than 1% of all donors [e.g., China: 0.4% (3,123) to 1% (140), Japan 0.5% (141)]. In China, about 24% of these donors have a Del phenotype (Japan, 9%; Korea, 15%; Thailand, 20%; percentages are median results of the surveys in *Table 6*). In 97% (China, Japan, Thailand) to 99% (Korea) of Del phenotypes the underlying allele is the Asian-type *RHD*01EL.01*. Hence, definition of the transfusion strategy for one single *DEL* allele, *RHD*01EL.01*, has major impact for transfusion support of patients typed D– by routine serology.

In contrast, in European populations, only 0.03% (Poland) to 0.28% (Croatia) of probands who are D– by routine serology possess a Del phenotype. Furthermore, the underlying alleles are heterogeneous and often not suitable for D+ transfusion. As a result, there is no advantage of identifying Del in patients. A possible exception may be patients with c or e negative phenotypes for whom Rh phenotype compatible D– blood is difficult to provide even in Europe. Still, the major Del issue in these populations is a possible anti-D immunization risk incurred by Del donors mistyped as D–.

As a corollary, it should be noted that the "European-derived" situation is not as uniform as it might seem: On closer inspection, relevant differences of the frequency of the "rare" *DEL* alleles can be detected. *RHD*01EL.08* is frequent in Central Europe [Austria (94), Denmark (62), Germany (20), Switzerland (79,93)]. *RHD*01EL.18* is found in francophone Canadians (121). *RHD*01EL.43* is dominant in Argentina (72) but otherwise rare. *RHD*11* is frequent in Central (20,54,63,76,79,93) and South-Eastern (116,128) Europe. *RHD*09.05* is frequent in parts of Austria (94) and in Brazil (119) in donors of African descent but seems to be rare elsewhere. In Austria, there is an obvious founder effect: Most carriers of *RHD*09.05* live near the river Traun (142) and possess a *RHCE*ce.20.13* allele. As *RHD*09.05* is one of the few Del types that can be found among seemingly D– C-E- samples, such local variation may be of importance for typing strategies.

Anti-D immunization

Anti-D immunization in Del patients

Anti-D immunization is unlikely in patients with the *RHD*01EL.01* allele (see paragraph "Asian-type *DEL*"). However, anti-D antibodies have been observed in patients with other *DEL* alleles (*Table 7*) (34,35,43,59,60,73,143).

Most of these anti-D were detected in pregnant females with hemolytic disease of the fetus or newborn, which can be severe (73). Anti-D immunizations have been reported for *RHD*01EL.04*, *RHD*01EL.08*, *RHD*01EL.30*, *RHD*01EL.31*, *RHD*01EL.44*, *RHD*01N.07*, *RHD*11* and *NL-8*. These alleles must therefore be considered partial Del. Three of them (*RHD*01EL.44*, *RHD*01N.07*, and *NL-8*) are *RHD/RHCE* hybrid alleles, two (*RHD*01EL.08* and *RHD*01EL.31*) carry splice site variations at position +1, the two remaining alleles are due to a frameshift in exon 1 (*RHD*01EL.04*) and the deletion of *RHD* exon 8 (*RHD*01EL.30*), respectively. Additional Del for which a partial Del phenotype has been predicted are *RHD*01EL.09*, *RHD*01EL.19*, *RHD*01EL.22*, *RHD*01EL.33*, *RHD*01EL.47*, and anti-D immunization is likely possible in carriers of these alleles. The same is true for carriers of alleles that are likely D– rather than Del.

The prediction of a partial Del phenotype based on its structure is sometimes difficult. Even the observation of an anti-D may be misleading: Even more than in weak D, the discrimination of an allo-anti-D from an auto-anti-D is difficult in Del: due to the low antigen density, a high titer auto-anti-D may be associated with a negative direct antiglobulin test. An example of auto-anti-D has been reported for *RHD*11* (81). Hence, the absence of an anti-D immunization risk in carriers of a specific *DEL* allele must be based on the observation of a large number of Del individuals. Despite several surveys (*Table 2*), convincing data are available only for *RHD*01EL.01* although the database is expected to vastly improve over time for the Asian-type and many other DEL alleles.

Anti-D immunization of D– patients by RBC units from Del donors

When the molecular investigation of seemingly D– blood donors revealed the presence of donors expressing D in the D– donor pool (20), the issue of possible anti-D immunization of D– patients by Del blood units was

Table 6 Population studies of *DEL*

Population	Positions screened[†]	Donors investigated	D– with C or E	D– donors screened	*DEL* detected	Frequency of *DEL* among D–	Causative allele (individuals[§])	Number of alleles[‡]/comments	Reference
Argentina	5'UTR, IVS 4, 3'UTR			1,314	6	1:219	*RHD*01EL.43* (n=5); *RHD*11* (n=1)		(80)
Argentina	5'UTR, IVS 4, 3'UTR [no explicit description but reference to (81)]		526		17	1:31	*RHD*01EL.43* (n=14); *RHD*01EL.08* (n=1); *RHD*01EL.44* (n=1); *RHD*01EL.26* (n=1)		(72)
Australia	4, 5, 10		2,027		37 (+8 *DEL alleles* among D–)	1:55	*RHD*01EL.01* (n=16 incl. 2 D–); *RHD*11* (n=9 incl. 1 D–); *RHD*01EL.08* (n=6 incl. 1 D–); *RHD*01EL.18* (n=4); *RHD*01EL.09* (n=4 incl. 3 D–); *RHD*01EL.05* (n=2); *"RHD*(ex8:del/CE)*"* (n=2); *RHD*01EL.21* (n=1); *RHD*01EL.22* (n=1); *RHD*01EL.48* (n=1); *RHD*01W.10* (n=1)	The molecular basis in the "*RHD*(*ex8:del/CE*)" probands was not fully resolved	(30)
Austria	5'UTR, 3, 10		738		7	1:105	*RHD*11* (n=5); *RHD*01EL.01* (n=1); *RHD*01EL.25* (n=1)		(63)
Austria (Upper Austria)	4, 7, 10			2,427	3	1:809	*RHD*01EL.08* (n=2); *RHD*09. 05* (n=1)		(88)
Austria (Upper Austria)	4, 7, 10			23,330	66	1:353	*RHD*09. 05* (n=31); *RHD*01EL.08* (n=24); *NL-3* (n=8); *RHD*01EL.04* (n=2); *RHD*01EL.01* (n=1); *RHD*01W.32* (n=1+1 weak D)		(94)
Bosnia-Herzegowina	3, 5, 10		92		4 (+5 weak D)	23	*RHD*11* (n=4);*RHD*01W.01* (n=2); *RHD*01W.03* (n=1); Unresolved (n=2)		(116)
Brazil					3		*RHD*01El.01* (n=2); *RHD*01EL.35* (n=1)	The algorithm used to identify the samples is not described	(117)
Brazil	IVS 4, 10			239	0	<1:80			(118)
Brazil	IVS 4, 7			2,450	10	1:245	*RHD*01EL.01* (n=5); *RHD*09. 05* (n=5)	*RHD*01EL.01* (n=5); *RHD*09. 05* (n=5)	(119)
Brazil	IVS 4, 10		520		4 (+14 weak D)		*RHD*01EL.01* (n=2); *RHD*11* (n=1); *RHD*01* (n=1)	Antigen density *RHD*01EL.01*: 22; *RHD*11*: 38; RHD: 35	(115)
Brazil	IVS 4, 7		405		6	1:68	*RHD*01EL.01* (n=3); *RHD*01EL.37* (n=2); *RHD*01EL.32* (n=1)	*RHD*01EL.01* (n=4); *RHD*01EL.37* (n=2); *RHD*01EL.32* (n=1)	(120)
Brazil	IVS 4, 7		517		3	1:172	*RHD*01EL.32* + x (n=1) *RHD*01EL.01*; *RHD*01EL.50*	(structure of *RHD*01EL.32*—like sample not finally resolved); *RHD*01EL.50* was not defined as Del in this study	(86)
Brazil	IVS 4, 7			1,403	1	1:1,403	*NL-9* (n=1)		(86)
Canada				3,980			*RHD*01EL.18* (n=7)	Allele frequency mentioned in the introduction	(121)
China	1, 4, 5, 6, 7			102	26		*RHD*01EL.01* (n=25); *RHD*01EL.36* (n=1)	*RHD*01EL.01* (n=34); *RHD*01EL.36* (n=1)	(15)
China	3, 4, 5, 6, 7, 9			74	22	1:3.4	*RHD*01EL.01* (n=22)	*RHD*01EL.01* (n=22)	(122)
China	Adsorption/elution; 3, 4, 5, 6, 7, 9	15,643 (Han: 12,546; Uigur: 1,814)		150 (Han: 50; Uigur: 86)	14 (Han: 11; Uigur: 2)	1:11 (Han: 1:4.5; Uigur: 1:43)	*RHD*01EL.01* (n=14)		(123)
China	Adsorption/elution	400,253		1,585	279	1:5.7	*RHD*01EL.01* (n=268); *RHD*01EL.02* (n=4); *RHD*01EL.44* (n=1); *RHD*01EL.03* (n=1); *RHD*01EL.06* (n=1); *RHD*01EL.07* (n=1); *RHD*01W.61* (n=1); *NL-6* (n=1); *NL-8* (n=1)		(3)
China (Han) Hefei	5'UTR, IVS 4, 7, 10			152	31	1:4.9	*RHD*01EL.01* (n=31)		(124)

Table 6 (*continued*)

Table 6 (*continued*)

Population	Positions screened[†]	Donors investigated	D– with C or E	D– donors screened	*DEL* detected	Frequency of *DEL* among D–	Causative allele (individuals[§])	Number of alleles[‡]/comments	Reference
China	Testing for *RHD*01EL.01*; *RHD* all exons			2,493	516	1:4.8	*RHD*01EL.01* (n=516)	*RHD*01EL.01* (n=565)	(24); overlap with (125)
China	Testing for *RHD*01EL.01*			2,385	516	1:4.6	*RHD*01EL.01* (n=516)	*RHD*01EL.01* (n=565)	(125); overlap with (24)
China	Serologic testing by adsorption/elution			165	41	1:4.0	*RHD*01EL.01* (n=37); *RHD*01EL.24* (n=1); *RHD*01N.07* (n=1); *NL-7* (n=1); *NL-8* (n=1)	*RHD*01EL.01* (n=39); *RHD*01EL.24* (n=1); *RHD*01N.07* (n=1); *NL-7* (n=2); *NL-8* (n=1)	(55)
China	Testing for *RHD*01EL.01*; serologic testing by adsorption/elution			643	155	1:4.1	*RHD*01EL.01* (n=151); other (n=4)		(33)
China	Serologic testing by adsorption/elution			808	178	1:4.5	*RHD*01EL.01* (n=158); *RHD*01EL.02* (n=8); *RHD*01EL.44* (n=1); *NL-8* (n=1)		(35)
China				804; subset: 515 pregnant women	221; subset: 142		Molecular analysis only for subset: *RHD*01EL.01* (n=130); *RHD*01EL.44* (n=7); *NL-8* (n=3); *RHD*01N.07* (n=1)		(34)
China (Taiwan)	RT-PCR and RFLP?			204	41		*NL-1* or *RHD*01EL.01* (n=41)	Allele reported as *NL-1* but data compatible with *RHD*01EL.01*	(126)
China (Taiwan)	serologic testing by adsorption/elution; 2, 3, 5, 7, 9			156	34	1:4.6	exon 2,3,5,7,9 detected (n=27: 26 C+E-, 1 C-E-); exon 2,3,5,7 detected (n=7; all C+E-)	structure of alleles not resolved beyond PCR pattern; PCR patterns observed in Del and D– difficult to reconcile with other studies	(127)
China (Taiwan)	Serologic testing by adsorption/elution; 4, 5, 7, 9, 10; Testing for RHD*01EL.01			294	94		*RHD*01EL.01* (n=94)	*RHD*01EL.01* (108)	(16)
China (Taiwan)	Testing for *RHD*01EL.01*; Adsorption/elution			395	130	1:3.0	*RHD*01EL.01* (n=126 + 1 D–); other (*4*)	One *RHD*01EL.01* sample was D–	(25)
China (Taiwan)	Serologic testing by adsorption/elution; Testing for *RHD*01EL.01*			118	38		*RHD*01EL.01* (n=38); + 1 *RHD*01EL.01*-like among D–		(18)
Croatia	Serologic testing with IAT			1,630	6	1:272	*RHD*11* (n=6)	Adsorption/elution only done in samples faintly positive in IAT; antigen density 28 to 44 (median 35)	(128)
Croatia	7, 10			6,523	12	1:544	*RHD*11* (n=4); *RHD*01* (n=4); *RHD*01W.02* (n=2); *RHD*01W.28* (n=1); *NL-10* (n=1)	*RHD*01W.02* and *RHD*01W.28* samples had a “C” in trans. The molecular cause of Del phenotype in *RHD*01* remained unresolved despite sequencing all exons	(99)
Denmark	10		233		3	1:78	*RHD*01EL.08* (n=2); *RHD*01EL.01* (n=1); *RHD*01EL.02* (n=1)		(62)
Denmark	5, 7, 10			5,058 (4,932 results available)	2	1:2,029	*RHD*01EL.33* (n=2); *RHD*01EL.08* (n=1 *D–*)	The *RHD*01EL.08* sample was considered D–	(53)
Finland	5, 7			16,253	5; +5 possible	1:3,250 (to 1:1,625)	*RHD*01EL.01* (n=2); *RHD*01EL.04* (n=1); *RHD*01EL.08* (n=1); *RHD*11* (n=1); possible *DEL: RHD*c.829G>A* (n=4); *RHD*c.[1154G>C;1163T>G]* (n=1)	“Possible Del”: negative in routine serology but molecular variation atypical for D–	(129)
Germany (South-West)	5'UTR, IVS 4, 7, 10		754		15 (+4 weak D/partial D)	1:50	*RHD*11* (n=7); *RHD*01EL.01* (n=5); *RHD*01EL.08* (n=3)		(20)

Table 6 (*continued*)

Table 6 (*continued*)

Population	Positions screened[†]	Donors investigated	D– with C or E	D– donors screened	*DEL* detected	Frequency of *DEL* among D–	Causative allele (individuals[§])	Number of alleles[‡]/comments	Reference
Germany (South-West)	5'UTR, IVS 4, 10			7,688	0	<1:2,500			(20)
Germany (North)	5'UTR, 3, 10		454		2	1:227	*RHD*11* (n=2)		(63)
Germany (South-West)	IVS 4			46,133	47	1:982	*RHD*01EL.08* (n=16); *RHD*11* (n=14); *RHD*01EL.01* (n=4); *RHD*01EL.04* (n=4); *RHD*01EL.18* (n=2); *RHD*01EL.25* (n=2); *RHD*01EL.12* (n=1); *RHD*01EL.16* (n=1); *RHD*01EL.17* (n=1); *RHD*01EL.23* (n=1); *RHD*01* (n=1)		(54)
India	Serologic testing by adsorption/ elution			200	3	1:67		2 Del C+, 1 Del E+	(130)
India	Serologic testing by adsorption/ elution; molecular testing for *RHD*01EL.01* and *RHD*11*			900	0	<1:243[$]			(131)
India	Serologic testing by adsorption/ elution			1,003	2	1:502		Both Del C+	(132)
Italy	Commercial PCR systems, pools of 5		235		1	1:235	*RHD*11* (n=1)		(133)
Japan	1, 2, 3, 4–6, 7, 8, 9, 10			3,526	324	1:11	*RHD*01EL.01* (n=318); *RHD*01* (n=3); *RHD*01EL.25* (n=2); *RHD*01EL.08* (n=1)	*RHD*01EL.01* (n=329); *RHD*01* (n=3); *RHD*01EL.25* (n=2); *RHD*01EL.08* (n=1)	(26)
Japan	1 to 10			2,754	240		*RHD*01EL.01* (n=232); *RHD*01EL.25* (n=2); *RHD*01EL.10* (n=1); *RHD*01EL.45* (n=1); *RHD*01N.60* (n=1); *RHD*01N.70* (n=1); Unresolved (n=2)		(11)
Korea	IVS 4, 7			126	16	1:7.9	*RHD*01EL.01* (n=16)	*RHD*01EL.01* (n=16)	(17)
Korea	3, IVS 4, 5,7,10			264	43	1:6.1	*RHD*01EL.01* (n=42); *RHD*01EL.10* (n=1)	*RHD*01EL.01* (n=42); *RHD*01EL.10* (n=1)	(27)
Korea	Promoter, IVS 4, 7, 10			110 ("D-negative club" members)	16	1:6.9	*RHD*01EL.01* (n=14); *RHD*01EL.10* (n=2)		(134)
Korea				95	17	1:5.6	*RHD*01EL.01* (n=17)		(135)
Korea	Promoter, IVS 4, 7, 10		50		27	1:1.9	*RHD*01EL.01* (n=26); *RHD*01EL.10* (n=1)		(136)
Morocco	Serologic testing by adsorption/ elution		425		4				(137)
Myanmar	Serologic testing by adsorption/ elution			222	35	1:6.3	No molecular characterization	9 weak D found	(138)
Netherlands	5, 7			37,782	34	1:1,111	*RHD*01EL.01* (n=9); *RHD*01EL.45* (n=7); *RHD*01EL.18* (n=6); *NL-4* (n=3); *RHD*(1-9)* (n=3); *RHD*01* (n=3); *RHD*01EL.11* (n=1); *RHD*01EL.17* (n=1); *RHD*01EL.46* (n=1)	*RHD*01EL.01* (n=9); *RHD*01EL.45* (n=7); *RHD*01EL.18* (n=6); *NL-4* (n=4); *RHD*(1-9)* (n=3) *RHD*01* (n=3); *RHD*01EL.11* (n=1); *RHD*01EL.17* (n=1); *RHD*01EL.46* (n=1)	(66)
Poland	IVS 4, 7, 10			31,200	10	1:3,120	*RHD*11* (n=5); *NL-6* (n=3); *RHD*01EL.08* (n=1); *RHD*01EL.32* (n=1)		(76)
Russia	5'UTR, 3, 10		71		0	<1:19[$]			(63)

Table 6 (*continued*)

Table 6 (*continued*)

Population	Positions screened[†]	Donors investigated	D– with C or E	D– donors screened	*DEL* detected	Frequency of *DEL* among D–	Causative allele (individuals[§])	Number of alleles[‡]/comments	Reference
Serbia	3, 5, 10		61		5	1:12	*RHD*11* (n=2); *RHD*15* (n=2); *RHD*01W.02* (n=1)		(116)
Slowenia	5'UTR, 3, 10		333		4	1:83	*RHD*01EL.01* (n=3); *RHD*11* (n=1)		(63)
Switzerland	5'UTR, 3, 10		104		2	1:52	*RHD*01EL.01* (n=2)		(63)
Switzerland (BTS Berne)	3, 5, 10			20,015	27	1:741	*RHD*11* (n=10); *RHD*01EL.08* (n=6); *RHD*01EL.46* (n=3); *RHD*01EL.01* (n=2); *RHD*01EL.18* (n=1); *RHD*01EL.41* (n=1); *RHD*01EL.42* (n=1); *RHD*01N.48* (n=1); *NL-2* (n=1); *RHD*01EL.43* (n=1)	Donors tested 2012; *RHD*11* and *RHD*01EL.43* weakly positive in IAT but considered *DEL*	(79); dataset possibly overlapping with (93)
Switzerland (BTS Zurich)	5,7,10			5,355	5	1:1,071	*RHD*11* (n=2) *RHD*01EL.08* (n=1); *NL-5* (n=2)	Donors tested 2012; *RHD*11* weakly positive in IAT but considered DEL	(79); dataset possibly overlapping with (93)
Switzerland (BTS Berne)	3, 5, 10		652		9	1:72	*RHD*11* (n=4); *RHD*01EL.08* (n=3); *NL-5* (n=2)		(93); dataset possibly overlapping with (79)
Switzerland (BTS Berne)	3, 5, 10			17,391	1	1:17,391	*RHD*01EL.08* (n=1)		(93); dataset possibly overlapping with (79)
Switzerland (BTS Zurich)	IVS 4, 7			8,200	10	1:820	*RHD*11* (n=5); *RHD*01EL.08* (n=4); *RHD*01EL.01* (n=1)		(93); dataset possibly overlapping with (79)
Thailand	(Serologic screening)			254	50	1:5.1	*RHD*01EL.01* (n=48); other (n=2)		(28)
Thailand	1 to 10			321	121	1:2.6	*RHD*01EL.01* (n=108+*21**); *RHD*01N.01* (n=11); *RHD*01N.03* (n=1); *RHD*15* (n=1)	*21 *RHD*01EL.01* alleles were found in "D-negative" samples, vice versa 11 "Del" samples were homozygous for the RHD deletion	(32)
Thailand	1 to 10			1,125	180	1:6.3	*RHD*01EL.01* (n=175); RHD* *01EL.44* (n=4); *RHD*01EL.08* (n=1). Three additional alleles were detected in a single sample and reported as D– without testing by adsorption/elution *RHD*c.325A>C*; *RHD*c.604G>A*; *RHD*c.[1136C>T;1223G>A]*	*RHD*01EL.01* (n=186); *RHD* 01EL.44* (n=5); *RHD*01EL.08* (n=1)	(29)
Tunisia	10			488	4 (incl. weak D/ partial D)	1:122	*RHD*11* (n=2); *RHD*09.03.01* (n=1); *RHD*01W.29* (n=1)		(139)
USA	Variant testing using beadchip assay			1,174	6	1:196	*RHD*01EL.01* (n=4); *RHD*01EL.08* (n=1); *RHD*01EL.09* (n=1)		(31)

[†]Numbers indicate exons tested by PCR. [§]Number of individuals with this allele (i.e., homozygous occurrence counted as 1). [‡]Number of alleles (i.e., homozygous occurrence counted as 2). Numbers only indicated if different to number of individuals. *In addition, an *RHD*01EL.08* sample was detected but characterized as D-negative. [$]Upper limit of 95% confidence interval (binomial distribution), estimated frequency 0. IVS, intervening sequence; UTR, untranslated region; incl., including; IAT, indirect antiglobulin test.

Table 7 Case reports of anti-D immunization in DEL patients

Allele	Observation	Reference
*RHD*01EL.04*	Anti-D observed in pregnancy	(60)
*RHD*01EL.08*	2 females with anti-D	(43)
*RHD*01EL.08*	2 mothers with anti-D; mild HDN in one child	(143)
*RHD*01EL.30*	Woman (2 stillbirth) with anti-D	(59)
*RHD*01EL.31*	Anti-D causing severe hemolytic disease in 2nd child	(73)
*RHD*01EL.44*	Anti-D observed in 3 of 7 individuals in survey	(34)
*RHD*01EL.44*	Anti-D observed in the single individual in survey	(35)
*RHD*01N.07*	Anti-D observed in the single individual in survey	(34)
NL-8	Anti-D observed in 2 of 4 individuals in survey	(34)
NL-8	Anti-D observed in the single individual in survey	(35)

raised immediately and a look back study was included in this publication. The first observation of an anti-D immunization caused by Del units was a female Austrian patient who was shown to have received an *RHD*01EL.35* unit (4). Shortly afterwards a considerable increase of the anti-D titer in a pre-immunized 67 years old Japanese woman receiving two *RHD*01EL.01* units was reported (144). The latter observation was important because *RHD*01EL.01* is the by far most frequent *DEL* allele in Asians (15-18).

Examples of possible anti-D immunization events caused by blood donations from Del blood donors are summarized in *Table 8* (4,11,97,121,144-150). Most reports concern *RHD*01EL.01*, which is not surprising since it is by far most frequent *DEL* allele.

Considering the many reports accrued, there is no doubt that Del RBC units may cause anti-D immunization. The evidence for primary anti-D immunization by Del is less strong: There are 8 reports suggesting a primary anti-D formation after transfusion of Del units, but in five of these, the anti-D occurred within one month. Although such rapid primary anti-D immunization is possible, it is suspicious: in most studies on D+ transfusion of D– probands, the anti-D occurred after 2 to 9 months with a mean of 17 weeks in one study (151,152). This finding was replicated in D– trauma patients after D+ transfusion in whom no anti-D formation was detected before 3 months and 8 of 9 anti-D were detected after more than 6 months (153). The "rapid" immunization by Del RBC units might be an observation bias (anti-D shortly after a D– transfusion is likely to trigger further investigations) but could also indicate that the assumed "primary" immunization represents a secondary immune response. In addition, in several reports, transfusion of D+ platelet units represents a possible alternative cause of anti-D immunization.

The likelihood of anti-D production has been targeted in several studies investigating the fate of patients transfused with Del RBC units (*Table 9*) (31,33,53,63,121,146,150). Usually only a few transfusions were informative: Many patients receiving Del RBC units were D+, had preexisting anti-D or were lost to follow-up. If only D– patients with follow up are considered, there were 103 informative transfusions among which 6 anti-D immunizations occurred. Four of these immunization events could alternatively have been caused by D+ platelet units. The risk of anti-D immunization after transfusion with "standard" D+ units is 20% to 50% depending on the patient population (151,152,154). An immunization risk of 4% to 7% would thus be about one tenth of the risk incurred by a normal D+ unit.

Some authors (53) suggest that such low immunization rate does not support general testing of blood donors for Del, especially in a population where avoiding C and E positive units is an alternative strategy (155). The situation is similar to weak D where cases of anti-D immunization by weak D units are well documented but this event is rare in prospective observations (156). Sometimes, the low risk of anti-D immunization is reinforced by comparing the immunization rates to those caused by other blood group antigens. Such comparisons miss an important point: despite the low immunogenicity of Del units, anti-D remains the leading cause of severe hemolytic disease of the

Table 8 Reports of immunization events caused by blood donations from blood donors with Del phenotype

Allele	Type	Sex	Age (years)	Observation	Reference
*RHD*01EL.01*	S	F	67	Two *RHD*01EL.01* units among 59 "D neg" units. Titer increase <1 to 8 after 1st unit; 8 to 128 after 2nd Del unit	(144)
*RHD*01EL.01*	P	M	68	1 *RHD*01EL.01* unit among 4 units transfused; anti-D 9 days after transfusion	(145)
*RHD*01EL.01*	P	M	68	2 *DEL* units -> new anti-D titer 2 after 22 days	(146)
	S	F	33	1 *DEL* unit -> titer increase 8 to 64	
	S	M	45	2 *DEL* units -> titer increase 8 to 64	
*RHD*01EL.01*	P	M	64	New anti-D 5 to 7 d after transfusion of 2 Del unit from 2 donors	(147)
*RHD*01EL.01*	P	F	54	new anti-D 2 month after transfusion of 1 *RHD*01EL.01* unit	(148)
*RHD*01EL.01 (probable)*	P	M	64	New anti-D 1 month after transfusion of 2 Del units	(149)
	S	M	73	New anti-D 6 days after transfusion of 4 Del units	
*RHD*01EL.01 (probable)*	S	F	70ies	"new" anti-D 34 days after 1 unit *RHD*01EL.01*	Compiled in (11)
	S	F	57	Titer <1 → 4096; first detected 2 years after 2 unit *RHD*01EL.01*	
	S	F	70ies	"new" anti-D + anti-C 28 days after 1 unit C+	
	S	F	85	Titer <1 → 8; first detected day 4 after 1 unit C+	
	P	M	35	New anti-D + Anti-C 5 months after 1 unit DEL	
	S	M	79	Titer <1 → 8, first detect 4 days after 1 unit *RHD*01EL.01*	
	S	F	86	New anti-D 9 days after 1 unit C+	
	S	F	80ies	Titer <1 → 2 2 weeks after 1 unit RHD*01EL.01	
*RHD*01EL.18*		F	87	Anti-D detected (see also *Table 9*)	(121)
		F	88	Anti-D detected (see also *Table 9*)	
		F	44	Anti-D detected (see also *Table 9*)	
		F	88	Anti-D detected (see also *Table 9*)	
*RHD*01EL.35*	P	F	58	New anti-D 8 days after transfusion of 1 *RHD*01EL.35* unit	(4)
NL-5	S	F	76	New anti-D 10 days after transfusion of 1 NL-5 unit	(97)
*RHD*01*	P?	M?	?	Anti-D 45 days after transfusion of 1 *DEL* unit; molecular analysis of the *DEL* detected no difference from *RHD*01*	(150)

P, primary immunization; S, secondary immunization; F, female; M, male; 70ies, age 70 to 79; 80ies, age 80 to 89; ?, not detailed or information not clearly described.

fetus in many populations (157,158) and preexisting anti-D-immunization, even if caused by a Del unit, makes anti-D prophylaxis futile. Thus, in many regions the harm caused by an anti-D immunization is more than that caused by an anti-E immunization.

An additional argument against the use of Del units for D– patients is a reduced hemoglobin increase after transfusion of Del units to patients with high titer anti-D observed by Shao *et al.* (146).

Some blood services started to identify Del donors by *RHD* PCR of blood donors [serologic testing is less suitable (58)]. Three approaches have been considered [also reviewed in (5)]:

(I) The most straightforward approach consists of testing all D– donors for Del. This algorithm is the "safest" one, as it does not depend on any assumptions regarding haplotype association. On the downside, it necessitates more testing than the

Table 9 Outcome of transfusion of blood donations from blood donors with Del phenotype

Survey	Country	Allleles	Anti-D*	Follow-up	Comment
Gassner *et al.* 2005 (63)	Austria	*RHD*01EL.11*	0/1	Not reported	
		*RHD*11*	0/7	17 to 145 days	
Krog *et al.* 2011 (53)	Denmark	*RHD*01EL.08*	0/11	<3 months (5) to >12 months (3)	D pos platelet units in 1 of 2 immunized patients
		*RHD*01EL.33*	2/29	<3 months (14) to >12 months (8)	
Shao *et al.* 2012 (146)	China	*RHD*01EL.01*	1/6	Not reported	3 additional patients with pre-existing anti-D: anti-D boostered in 2 of 3; insufficient Hb increase
St-Louis *et al.* 2013 (121)	Canada	*RHD*01EL.18*	3/31		D pos platelet units
		*RHD*11*	0/1		
Wang *et al.* 2014 (33)	China	*RHD*01EL.01*	0/14	Not reported	
Perez-Alvarez *et al.* 2019 (31)	USA	*RHD*01EL.01*	0/1	37 days	
		*RHD*01EL.08*	0/2	711 to 729 days	
Safic Stanic *et al.* 2022 (150)	Croatia	*RHD*01*	1/?	Not reported	The number of informative transfusions is not reported per allele. In total, there were 40 informative D– recipients
		*RHD*11*	0/?		
		*RHD*01W.2*	0/?		
		*RHD*01W.28*	0/?		
		NL-10	0/?		

*Number of observed anti-D immunization and number of D– patients without anti-D prior to transfusion transfused and not lost to follow up. ?, data not disclosed in the reference.

other algorithms. This algorithm is obligatory in Switzerland (79), listed as possible approach in the German transfusion guidelines (159) and has been implemented by several blood services in Austria (94), Germany (54) and the United States (31). As prices for molecular techniques are generally decreasing while those for serologic testing tend to increase, it might even become cost efficient if it replaces sensitive serologic testing for D in blood donors (5). Still, the costs reported for 2012 in Switzerland were between 7 and 10 € per sample investigated (79) which is considerably higher than serologic testing by IAT.

(II) Since the vast majority of *DEL* alleles occur in a DCe or DcE haplotype, algorithms have been proposed that limit testing for Del to D–, C+ probands (25,28,58,134) or to D– probands whose RBC express C or E. In many countries, determination of the full Rh phenotype is routine and such algorithm may reduce testing considerably. This approach has been advocated in China (including Taiwan) (25), Korea (134), and Thailand (28). It should be noted, however, that the algorithm will fail in populations in which *RHD*09.05* or other ce-associated *DEL* alleles are frequent, like Austria and Argentina. Furthermore, there are rare observations of *RHD*01EL.01* in C negative probands (15).

(III) Finally, it has been discussed that transfusion patients at high risk of anti-D immunization exclusively with D– RBC units lacking antigen C (11) or lacking antigens C and E (155) might be an alternative to testing for Del. Obviously, such strategy is easier in countries with plenty of D– units lacking C and E than in East Asian populations where D– units are scarce and often

carry antigen C.

Conclusions

Much progress has been made in defining the molecular basis of Del and the serological and clinical implications of this phenotype. More than 45 distinct *DEL* alleles are recognized by the ISBT, ten additional alleles might also qualify as *DEL*. The most frequent mechanisms leading to DEL alleles are missense variations and variations interfering with splicing. Less frequently, hybrid alleles, frameshift variations, premature termination codons and large deletions encompassing whole exons are observed. The current knowledge has relevant limitations: Some variations observed in *DEL* alleles cannot influence the phenotype and in some Del samples no variations were detected. Technical confounders include an ill-defined boundary between weak D and Del as well as false-positive and false-negative adsorption/elution results impeding the serologic discrimination of Del from D negative. Similar to *weak D* alleles, distinct *DEL* alleles display distinct serologic profiles that differ in the RhD antigen density, the absence of D epitopes and a possible anti-D immunization risk in carriers of these *DEL* alleles. The prevalence of *DEL* alleles varies widely between populations, resulting in different implications for transfusion strategies: In East Asian populations, a relevant proportion of individuals D– in routine serological D testing possess the *RHD*01EL.01* allele. Hence, in these populations, definition of the correct transfusion strategy for patients with *RHD*01EL.01* has relevant impact on the D– blood supply, even more than the D+ transfusion strategy for the frequent weak D types in Europe. In contrast, in many other populations, Del is a rarity and caused by numerous different alleles. In such populations, the main focus is the prevention of anti-D immunization of D– patients by Del blood units released as D negative.

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