A comprehensive analysis of DEL types: partial DEL individuals are prone to anti-D alloimmunization

Günther F. Körmöczi, Christoph Gassner,* Chao-Peng Shao, Makoto Uchikawa, and Tobias J. Legler*

BACKGROUND: The D antigen of the polymorphic Rh blood group system is of particular clinical importance regarding transfusion- and pregnancy-induced alloimmunization. Different RhD variants with specific clinical implications have been characterized. The least expressed D variants collectively called DEL are serologically detectable only by adsorption-elution techniques, with so far only poorly defined antigenic properties.

STUDY DESIGN AND METHODS: A comprehensive immunohematologic analysis of five of the six currently known *DEL* genotypes was performed. DEL phenotypes associated with the *RHD*(M295I), *RHD*(IVS3+1g>a), *RHD*(K409K), *RHD*(X418L), or *RHD*(IVS5-38del4) allele were characterized with extended serology and flow cytometry.

RESULTS: Epitope mapping with adsorption-elution revealed a prominent D epitope loss in the *RHD*(IVS3+1g>a)-associated DEL phenotype, whereas in the other four DEL types no signs of qualitative D antigen alteration were detected. The observation of alloanti-D in two *RHD*(IVS3+1g>a) cases confirmed the partial nature of this DEL phenotype. The *RHD*(M295I) phenotype exhibited the highest D antigen expression among all investigated DEL types, as determined by a semiquantitative adsorption-elution approach and flow cytometry.

CONCLUSION: In conclusion, evidence is provided that different *DEL* genotypes code either for partial or complete D antigen expression and that this finding is clinically relevant.

The Rh blood group system is determined by the highly homologous *RHD* and *RHCE* genes encoding the RhD and RhCE polypeptides, respectively. The D antigen (RH1) carried by the RhD polypeptide is of particular clinical importance with respect to transfusion- and pregnancy-induced alloimmunization.[1] Numerous RhD variants have been characterized so far, both on the serologic and the molecular level. Weak D-type red blood cells (RBCs) express reduced numbers of D antigens as a consequence of *RHD* point mutations with predicted amino acid substitutions in intracellular RhD portions,[2] whereas partial D variants with characteristic D epitope loss are caused by either *RHD-CE-D* hybrid genes or *RHD* point mutations affecting extracellular RhD loops.[3] The distinction between weak and partial D is of clinical importance, because only partial D individuals exhibit a tendency to anti-D alloimmunization upon challenge with normal D antigen consequent to their genetically determined D antigen alteration.[1,4,5]

ABBREVIATION: SSP = sequence-specific priming.

From the Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria; the Central Institute for Blood Transfusion and Immunological Department, General Hospital and University Clinics Innsbruck, Innsbruck, Austria; the Shenzhen Institute of Transfusion Medicine, Shenzhen, PR China; the Tokyo Blood Center, Japanese Red Cross, Tokyo, Japan; and the Department of Transfusion Medicine, University of Göttingen, Göttingen, Germany.

Address reprint requests to: Günther F. Körmöczi, Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria; e-mail: guenther.koermoeczi@meduniwien.ac.at.

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*G.F.K and C.G. contributed equally to this study.

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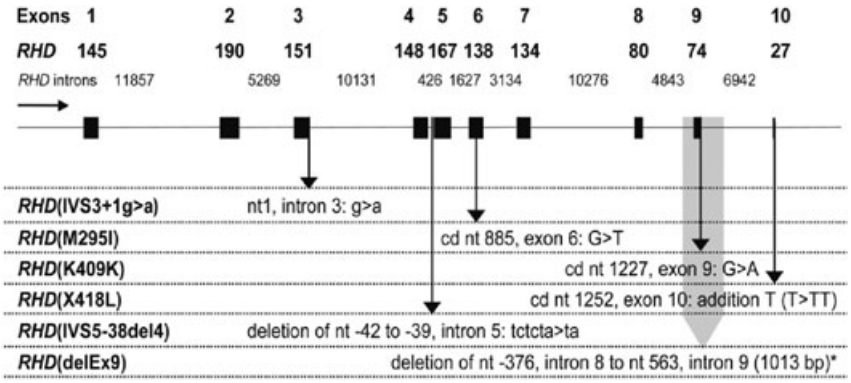

Fig. 1. Molecular background of known DEL alleles. An in-scale scheme of the *RHD* gene is depicted, with introns shown at 1/10th of their length. Exon count and exon and intron length in base pairs (bp) are given. Arrows connect the graphic representation of the *RHD* gene mutations with the respective *DEL* allele description. For *RHD*(delEx9), the large deletion encompassing exon 9 is indicated by a gray arrow-bar. *RHD*(delEx9) is the only described *DEL* allele not investigated in this study. Letters to the left of ">" denote the nucleotide (nt) sequence of the regular *RHD* gene, to the right of ">" the one of the mutated *DEL* allele. Nucleotides given in small letters indicate intronic sequences, and those given in capitals represent coding (cd) sequences. *The 5′ position of the break point for the 1013-bp deletion in *RHD*(delEx9) could not be identified by us with the published nucleotide sequences.

The weakest D variants collectively called DEL appear D– even when anti-D typing is performed with a sensitive indirect antiglobulin test (IAT). D antigen expression by DEL RBCs is serologically detectable only by adsorption-elution techniques.[6] DEL variants derive from *RHD* missense or splice-site mutations[7-9] or a deletion of *RHD* exon 9.[10] A total of six different *DEL* alleles were described so far (Fig. 1). *RHD*(M295I) seems to be the most frequent in Caucasian persons, occurring with a reported frequency of up to 1:3700 individuals in different European populations.[7,9] *RHD*(IVS3+1g>a) is the second allele that has only been observed in Caucasian persons until now with a frequency of up to 1:1800 individuals.[7,9] The prevalence of *RHD*(K409K) in Han Chinese and *RHD*(delEx9) in Taiwanese individuals suggests both alleles as "Asian," although *RHD*(K409K) has also been observed in Germany.[7-11] The remaining two *DEL* alleles, *RHD*(X418L) and *RHD*(IVS5-38del4), were very recently discovered in Austria.[9,12]

DEL individuals are routinely typed D–; however, it is questionable if this classification is safe for all molecular types and in every clinical situation. We recently reported the first case of anti-D immunization in a D– recipient owing to the transfusion of a RBCs from a *RHD*(IVS5-38del4) DEL donor.[12] In addition, also secondary anti-D immunization by *RHD*(K409K) RBCs was observed more recently (H. Yasuda et al., submitted for publication). In this study, a comprehensive analysis of the immunohematologic properties of all known DEL types, except for *RHD*(delEx9), which was not available for this study, was performed. A new concept is presented distinguishing between DEL types expressing apparently conserved D antigens versus a partial DEL with detectable D epitope loss. The clinical relevance of such classification is emphasized by two DEL cases with documented anti-D alloimmunization.

MATERIALS AND METHODS

Studied DEL individuals

A blood sample from a 21-year-old woman (Table 1, Proband 1) typed D– in IAT, with anti-D immunization, was referred to our laboratory for further investigation because she could not report any pregnancy or previous blood transfusions. After *DEL* allele identification in this case, an additional 15 blood samples representing all known *DEL* alleles except for *RHD*(delEx9) were selected to compare the antigenic properties of different molecular *DEL* types. These samples have been identified through molecular screening with PCR with sequence-specific priming (SSP) of individuals with D– IAT results (Table 1, Probands 2-12)[8,9] or in the course of a lookback analysis in a D– patient who developed anti-D after RBC transfusion (Table 1, Probands 13-16).[12] All individuals were C+c+E–e+ and were genotyped *CcDdee*. D antigen densities of individuals 13 through 16 and a part of the reaction pattern of RBCs from Individual 13 with different anti-D monoclonal antibodies (MoAbs) have been published previously and are reported here only for comparison.[12]

Molecular biology

Genomic DNA from Proband 1 was isolated from ethylenediaminetetraacetate-anticoagulated venous blood. We used commercially available PCR-SSP kits for *RHD* and *RHCE* genotyping and screening for *RHD* variants (RH-Type, partial D-type, weak D-type, D zygosity type, BAG, Lich, Germany). Nucleotide sequencing of *RHD* exons 1 through 10 from genomic DNA with adjacent intron sequences was performed with intron primers as described previously.[13] Details of the molecular *DEL* allele characterization of Individuals 2 through 16 have been reported previously.[8,9,12]

Immunohematology

Typing for Rh antigens D, C (RH2), E (RH3), c (RH4), and e (RH5) was performed by standard plate, tube, and gel

TABLE 1. Investigated DEL individuals

Proband*	Sex (male/female)	Age (years)	Origin	Ethnic group	DEL allele
1	Female	21	Germany	Caucasoid	*RHD*(IVS3+1g>a)
2	Male	65	Austria	Caucasoid	*RHD*(IVS3+1g>a)
3	Female	38	China	Asian	*RHD*(K409K)
4	Female	36	China	Asian	*RHD*(K409K)
5	Female	30	China	Asian	*RHD*(K409K)
6	Female	24	Austria	Caucasoid	*RHD*(X418L)
7	Male	54	Austria	Caucasoid	*RHD*(M295I)
8	Male	56	Austria	Caucasoid	*RHD*(M295I)
9	Female	60	Austria	Caucasoid	*RHD*(M295I)
10	Male	20	Austria	Caucasoid	*RHD*(M295I)
11	Male	56	Austria	Caucasoid	*RHD*(M295I)
12	Male	33	Austria	Caucasoid	*RHD*(M295I)
13	Male	31	Austria	Caucasoid	*RHD*(IVS5-38del4)
14	Female	25	Austria	Caucasoid	*RHD*(IVS5-38del4)
15	Male	52	Austria	Caucasoid	*RHD*(IVS5-38del4)
16	Male	8	Austria	Caucasoid	*RHD*(IVS5-38del4)

* Except for the four family members with the *RHD*(IVS5-38del4) allele, all DEL individuals were unrelated.

matrix techniques (Micro Typing System, DiaMed, Cressier, Switzerland). Blended monoclonal anti-D reagents (DiaClon anti-D, DiaMed and Seraclone anti-D blend, Biotest, Dreieich, Germany) were used for direct agglutination. Weak D detection was performed by IAT in gel matrix (DiaMed), as was the direct antiglobulin test (DAT) with monospecific reagents (anti-immunoglobulin G [IgG], -IgA, -IgM, -C3c, -C3d). Specification of unexpected RBC antibodies was performed with untreated test RBC samples (at 4 and 37°C in neutral gel and anti-human globulin gel cards, respectively) and bromelin-treated cells (at 37°C in anti-human globulin gel cards). Antibody titration was performed in tubes with polyspecific anti-human globulin (Ortho, Neckargemünd, Germany).

In all investigated samples the DEL phenotype was confirmed by adsorption of blended monoclonal anti-D (DiaClon anti-D and Seraclone anti-D blend) onto DEL RBCs in parallel with *ccddee* and *CcDdee* control RBCs at 37°C, extensive washing with cold phosphate-buffered saline (PBS), and subsequent acid elution (DiaCidel, DiaMed) for further antibody specification. For D epitope mapping of different DEL types, essentially the same adsorption-elution procedure was performed as previously described,[12] with a panel of single human anti-D MoAbs (clones P3×249, P3×290, P3×35, P3×241, HM16, P3×61, HM10, P3×212-11F1, P3×212-23B10 [Diagast, in part donated by K. Göttfert]); ESD1 and LHM70/45 (provided by A. Köchli, DiaMed); BRAD-3 and BRAD-5 (provided by G. Daniels, Bristol Institute for Transfusion Sciences, Bristol, UK); and Birma D6 (International Blood Group Reference Laboratory, Bristol, UK). Finally, eluates from IgG and IgM MoAb adsorptions were assayed for reactivity with a panel of one D– and two D+ RBC samples in anti-human globulin gel cards (DiaMed).

Flow cytometry

D antigen density of DEL and control RBCs was determined by flow cytometry (FACSCalibur, Becton Dickinson, Heidelberg, Germany) as described.[12,14] Briefly, 50 µL of washed RBCs in PBS was incubated at 37°C for 30 minutes with 50 µL of anti-D IgG MoAbs BS228, BS231 (provided by M. Ernst, Biotest), P3×290, P3×35, P3×241, P3×249, HM16, BRAD-3, BRAD-5, Birma D6, or negative control MoAb (AEVZ5.3, IBGRL). Fluorescein isothiocyanate–conjugated goat anti-human IgG Fab fragments (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) served as secondary reagent. Absolute D antigen densities (D antigens per RBC) were assessed by use of a standard *CcDdee* RBC sample with 9748 D sites per RBC.[5] For D antigen density calculation, the recommended algorithm was applied.[14] The lower limit of D antigen density detection was defined as the mean of the median fluorescence intensities of 15 *ccddee*– control RBC samples obtained with an individual anti-D MoAb plus two times its standard deviation (SD) as previously reported.[12]

Expression analysis of Rh antigens other than D and Rh-associated molecules was performed as described[12] by use of human polyclonal anti-C (Molter, Neckargemünd, Germany), anti-c (DiaMed), anti-e (Molter), and murine MoAbs against anti-LW^{ab} (BS46, provided by M. Ernst, Biotest), Rh-associated glycoprotein (RhAG; 2D10, provided L. de Jong, Sanquin, Amsterdam, the Netherlands), total Rh polypeptide (Rh30, RhD/RhCE; Bric69, IBGRL), and CD47 (B6H12, BD Biosciences, San Diego, CA). R-phycoerythrin–conjugated goat anti-human IgG $F(ab')_2$ (Immunotech, Marseille, France) or rabbit anti-mouse IgG $F(ab')_2$ (Dako Cytomation, Glostrup, Denmark) served as secondary reagents. Mouse IgG-negative controls were from Dako.

RESULTS

Molecular biology

Molecular characterization of Individual 1 with PCR-SSP revealed the presence of all tested *RHD* exons (exons 2-7). Furthermore, a *CcDdee* genotype was determined, and weak D types 1 through 5, 11, and 15 were excluded. Nucleotide sequencing of *RHD* exons 1 through 10 identified a *RHD*(IVS3+1g>a) allele. The molecular characteristics of Samples 2 through 16 were published previously, as mentioned under Materials and Methods.

Serology

All investigated DEL samples were found to be negative with routine monoclonal and polyclonal anti-D reagents in tube and gel matrix IAT. Acid eluates prepared from *RHD*(K409K), *RHD*(IVS5-38del4), *RHD*(X418L), and *RHD*(M295I) RBC samples after adsorption with routine anti-D blends DiaClon (anti-D MoAb clones MS26 and TH28) or Seraclone (clones BS221, H41 11B7, and BS232) were all reactive. In contrast, after separate adsorption of these two reagents onto *RHD*(IVS3+1g>a) RBCs, only DiaClon but not Seraclone anti-D could be eluted, indicating that this DEL type may be partial in nature. Moreover, the strength of reactivity of eluted anti-D (DiaClon) with D+ test RBCs in gel matrix IAT differed among DEL types, with 1+, 2+, 2+, 2 to 3+, and 3 to 4+ positive reactions when eluted from *RHD*(IVS3+1g>a), *RHD*(K409K), *RHD*(X418L), *RHD*(IVS5-38del4), and *RHD*(M295I) RBCs, respectively. This suggested further differences in D antigen expression between different DEL types.

Two individuals with the *RHD*(IVS3+1g>a) allele had a positive antibody screen, with anti-D specificity in both cases as determined by use of 21 D+ and 20 D– test RBC samples in IAT. Enhanced antibody reactions were observed with enzyme-treated test cells, and the two serum samples did not agglutinate RBCs at 4°C. Anti-LW specificity was ruled out in both cases, because dithiothreitol treatment of panel cells did not attenuate positive reactions. The anti-D titers against *ccDDEE* RBCs in tube technique IAT were 32 and 4 in the female and male *RHD*(IVS3+1g>a) individuals, respectively. All DEL samples had a negative DAT, and eluates prepared after DEL RBC incubation with autologous plasma did not react. The latter finding indicated that the anti-D encountered in the two *RHD*(IVS3+1g>a) individuals was an alloantibody. The female anti-D carrier (Proband 1) denied any prior transfusions or pregnancy, whereas the male individual (Proband 2) had received numerous RBC units of unknown antigen profile because of polytrauma more than three decades ago.

Different DEL alleles are associated with either partial or complete D antigen expression

Because of the obvious anti-D alloimmunization in both *RHD*(IVS3+1g>a) individuals, a detailed examination of the D antigen expressed by different DEL phenotypes was performed. Adsorption-elution–based epitope mapping with different anti-D MoAbs revealed that the *RHD*(IVS3+1g>a) allele was associated with a partial D antigen, devoid of a major proportion of normal D epitopes (Table 2).[15] In contrast, *RHD*(K409K), *RHD*(IVS5-38del4), *RHD*(X418L), and *RHD*(M295I) were found to express all tested D epitopes, suggesting the expression of a complete D antigen.

D antigen densities of different DEL phenotypes

To quantify the D expression across different DEL phenotypes, the absolute numbers of D antigens per RBC were assessed by flow cytometry.[12] The DEL phenotype associated with the *RHD*(M295I) allele was observed to express the highest numbers of D sites per RBC (Table 3). In five

TABLE 2. Reactivity pattern of DEL RBCs with anti-D MoAbs as assessed by adsorption-elution techniques

Anti-D MoAb	D epitope*	*RHD*(IVS3+1g>a) (n = 2)	*RHD*(K409K) (n = 3)	*RHD*(IVS5-38del4) (n = 2)	*RHD*(X418) (n = 1)	*RHD*(M295I) (n = 4)
LHM70/45	1.2	–	+	+	NT†	+
P3×249	2.1	Weak +	+	+	+	+
P3×290	3.1	–	+	+	+	+
ESD1	4.1	–	+	+	+	+
P3×35	5.4	–	+	+	Weak +	+
P3×241	5.4	+	+	+	+	+
BRAD-3	6.2	–	+	+	+	+
HM16	6.4	–	+	+	+	+
P3×61	6.4	–	+	+	+	+
HM10	6.6	–	+	+	+	+
BRAD-5	6.8	–	+	+	+	+
P3×212-11F1	8.2	–	+	+	+	+
P3×212-23B10	9.1	–	+	+	+	+
Birma D6	9.1	–	+	+	+	+

* D epitope nomenclature according to M. Scott.[15]
† NT = not tested.

TABLE 3. D antigen densities of different DEL and two control RBC samples

RBC sample	Number of D sites per cell
RHD(IVS3+1g>a)	
Sample 1	<22
Sample 2	<22
RHD(K409K)	
Sample 4	<22
Sample 5	<22
RHD(X418L)	
Sample 6	<22
RHD(M295I)	
Sample 7	33
Sample 8	29
Sample 9	34
Sample 10	36
Sample 11	<22
Sample 12	34
RHD(IVS5-38del4)*	
Sample 13	<22
Sample 14	26
Sample 15	<22
Sample 16	<22
Weak D type 1 *CcDdee* control	992
CcDdee control	10,429

* D antigen densities of all *RHD*(IVS5-38del4) individuals were published earlier[12] and are shown here only for comparison.

of the six analyzed *RHD*(M295I) individuals, specific fluorescence intensities with indirect anti-D staining were found to be above the defined lower limit of detection (i.e., the mean of the fluorescence intensities obtained with *ccddee*– control RBCs plus two times the respective SD, corresponding to a D antigen density of 22 per cell), which allowed for the calculation of absolute D densities. In a previous study,[12] only in one of four family members with the *RHD*(IVS5-38del4) phenotype an absolute D antigen density could be established. *RHD*(IVS3+1g>a), *RHD*(X418L), and *RHD*(K409K) samples exhibited fluorescence intensities below the defined detection threshold. These flow cytometric results paralleled the graded reactivity of eluates prepared from DEL RBCs adsorbed with routine anti-D reagents, as detailed earlier. Taken together, different DEL types exhibited not only qualitative but also quantitative D antigen differences.

Expression of Rh membrane complex molecules by DEL variants

The haplotypic *RH* gene constellation is known to individually impact not only on D but also on C, c, E, and e antigen expression.[1,16] Moreover, *RHAG* mutations are responsible for the regulator type Rh_{null} and Rh_{mod} phenotype,[17,18] and RhAG (Rh50, CD241) was shown to posttranscriptionally influence RhD expression.[19] Rh_{null} RBCs were found to have markedly diminished CD47 (integrin-associated protein) and absent LW (CD242, intercellular

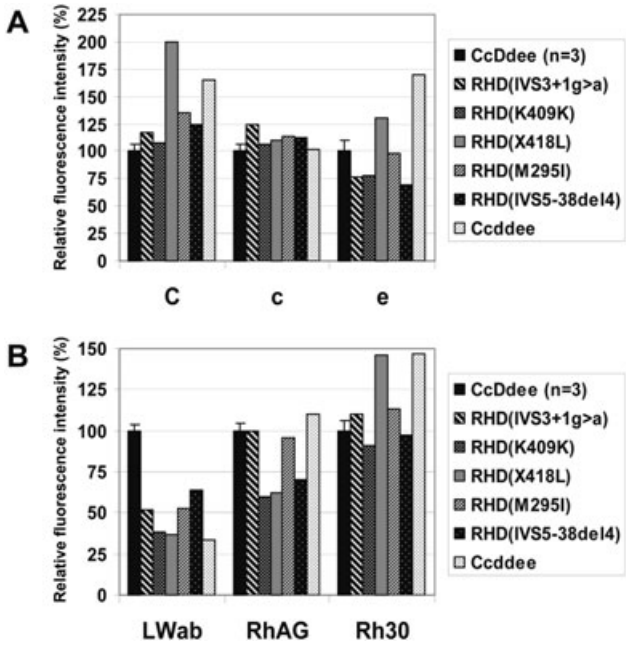

Fig. 2. Rh antigen and Rh membrane complex expression of DEL and control RBCs. Relative fluorescence intensities of different DEL type and *Ccddee* control RBCs indirectly stained with (A) anti-C, anti-c, and anti-e, and (B) anti-LW^{ab}, anti-RhAG, and anti-Rh30 are compared to averaged values (mean and SD) of three *CcDdee* control samples set as 100 percent.

adhesion molecule 4) expression. In addition, LW expression is higher in newborns than in adults and more pronounced in D+ individuals than D– individuals.[1]

These mutual influences of different Rh membrane complex molecules prompted us to analyze their expression in the five DEL types under study. We observed a tendency toward higher C antigen expression in DEL-type RBCs compared to *CcDdee* controls, with similar c and heterogenous e antigen expression (Fig. 2A). The LW^{ab} levels of DEL types were generally diminished compared to *CcDdee* controls, assuming values close to *Ccddee* control (Fig. 2B). DEL RBCs exhibited a tendency toward decreased and increased RhAG and total Rh polypeptide (Rh30) expression, respectively, compared to *CcDdee* RBCs (Fig. 2B). A highly uniform CD47 expression was observed in DEL and control RBCs (data not shown).

DISCUSSION

So far, no reports were available describing DEL individuals with partial D antigen expression. In one Japanese case report, a DEL woman who developed anti-D (titer 8 in IAT) during pregnancy was described.[20] She gave birth to two D+ children who presented no signs of hemolysis. Except for this single case devoid of molecular DEL specification, we could not find any report of anti-D alloimmunization in DEL individuals in the English, German, Chinese, or

Japanese literature. Furthermore, in two Japanese studies with 45 and 17 cases with anti-D, respectively, no DEL type was detected despite the fact that this phenotype is relatively frequent among individuals from the Far East routinely typed D–.[21,22] Throughout the world, the vast majority of DEL phenotypes are misinterpreted as D– owing to the limits of routine typing. For this reason, anti-D alloimmunization in DEL individuals may in fact not be a very rare event, but instead the DEL phenotype may be mostly unrecognized. A predisposition to alloanti-D formation requires D epitope loss and may therefore likely be DEL type-specific.

In this study, five of the six known *DEL* types were investigated. The sixth *DEL* type, *RHD*(delEx9), was not available for this study because this type has never been observed in our sample collective and was not available from the source of original observation in Taiwan. Checking the published nucleotide sequences of *RHD*(delEx9), the given 5′ breakpoint of the 1013-bp deletion located in intron 8 of the *RHD* gene could not be positively identified by us, when compared to known genomic sequences of *RHD* (GenBank Accession Numbers BX640519 and AL928711). Moreover, in a recent study the *RHD*(K409K) allele was found to be the exclusive cause of the DEL phenotype in Taiwanese blood donors.[23] Therefore, a molecular reevaluation of the *RHD*(delEx9) allele might probably be of advantage.

Nevertheless, in this study all other five DEL phenotypes were compared regarding D epitope expression, absolute D antigen density, and expression of Rh membrane complex molecules. Adsorption-elution procedures with a panel of anti-D MoAbs with known D epitope specificity demonstrated a prominent qualitative D antigen alteration in individuals with the *RHD*(IVS3+1g>a) allele, whereas the other four DEL types expressed basal levels of D antigens without apparent signs of D epitope loss. In accordance with these results, anti-D alloimmunization was only found in two *RHD*(IVS3+1g>a) individuals. A possibly unnoticed D+ pregnancy could have accounted for the anti-D alloimmunization in the female anti-D carrier. Despite a positive transfusion history, however, no definitive cause for anti-D induction in Proband 2 could be elucidated; it could be assumed that he had received only D– RBC units in the past, because most DEL samples are routinely typed D–. An additional three adult *RHD*(IVS3+1g>a) individuals (one woman, two men) from Slovenia identified in the course of a previous study[9] had a negative antibody screen. No information on transfusion history or pregnancy of the latter individuals was available. Taken together, a considerable proportion of the *RHD*(IVS3+1g>a) individuals with known antibody status were anti-D alloimmunized.

Different DEL phenotypes feature not only qualitative but also quantitative differences as indicated by the results of the semiquantitative adsorption-elution approach and the flow cytometric investigation. The absolute D antigen density of more than half of the DEL samples was estimated to be less than 22, with a maximum number of 36 D sites per cell in one sample. This is compatible with a recent report on weak D type 26, where a sample with 70 D sites per RBC was reliably typed D+ with all routine anti-D reagents in gel matrix IAT, whereas another sample with 29 D sites typed D+ only with some but not all reagents.[9] Similarly, either DEL or weak D phenotypes were observed to be associated with *RHD*(K409K) and *RHD*(885T) alleles.[7,8] These examples suggest that weakly expressed D variants are within a continuous spectrum from weak or partial D to complete or partial DEL.

Sensitive D antigen detection is of particular importance for donor typing;[24] however, current routine serology may at times be insufficient for the recognition of extremely weak D variants. Although weakly expressed D antigens were presumed to be of minor immunogenicity,[25] weak D type 2, weak D type 26, and in two cases even *RHD*(IVS5-38del4) and *RHD*(K409K) DEL RBCs were reported to induce anti-D in D– transfusion recipients (H. Yasuda, et al., submitted for publication).[9,12,26] D variants without D epitope loss are probably more immunogenic than those expressing similar numbers of partial D antigens. In any case, the combined use of serologic and molecular D typing techniques may reduce the number of such transfusion incidents.

In conclusion, we propose a novel concept of partial DEL versus complete DEL phenotypes. Such segregation is of clinical importance, as is indicated by the fact that both investigated partial DEL individuals were found to be anti-D alloimmunized. Numerous examples of severe to fatal hemolytic disease of the newborn (HDN) caused by anti-D in partial D women have been reported,[27-32] with clinical manifestations indistinguishable from HDN caused by anti-D in D– women.[33] It can be assumed that alloanti-D formed by partial DEL individuals may have the same deleterious potential with respect to HDN and may also trigger hemolytic transfusion reactions.

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REFERENCES

1. Daniels G. Human blood groups. 2nd ed. Oxford: Blackwell Science; 2002.
2. Wagner FF, Gassner C, Müller TH, et al. Molecular basis of weak D phenotypes. Blood 1999;93:385-93.
3. Avent ND, Reid ME. The Rh blood group system: a review. Blood 2000;95:375-87.

4. Mayne KM, Allen DL, Bowell PJ. "Partial D" women with anti-D alloimmunization in pregnancy. Clin Lab Haematol 1991;13:239-44.
5. Körmöczi GF, Legler TJ, Daniels GL, et al. Molecular and serologic characterization of DWI, a novel "high-grade" partial D. Transfusion 2004;44:575-80.
6. Okubo Y, Yamaguchi H, Tomita T, Nagao N. A D variant, Del? Transfusion 1984;24:542.
7. Wagner FF, Frohmajer A, Flegel WA. RHD positive haplotypes in D negative Europeans. BMC Genet 2001;2:10.
8. Shao CP, Maas JH, Su YQ, et al. Molecular background of Rh D-positive, D-negative, Del and weak D phenotypes in chinese. Vox Sang 2002;83:156-61.
9. Gassner C, Doescher A, Drnovsek T, et al. Presence of RHD in serologically D-, C/E+ individuals: a European multicenter study. Transfusion 2005;45:527-38.
10. Chang JG, Wang JC, Yang TY, et al. Human RhDel is caused by a deletion of 1,013 bp between introns 8 and 9 including exon 9 of RHD gene. Blood 1998;92:2602-4.
11. Peng CT, Shih MC, Liu TC, et al. Molecular basis for the RhD negative phenotype in chinese. Int J Mol Med 2003;11:515-21.
12. Wagner T, Körmöczi GF, Buchta C, et al. Anti-D immunization by DEL red blood cells. Transfusion 2005;45:520-6.
13. Legler TJ, Maas JH, Köhler M, et al. RHD sequencing: a new tool for decision making on tranfusion therapy and provision of Rh prophylaxis. Transfus Med 2001;11:383-8.
14. Flegel WA, Curin-Serbec V, Delamaire M, et al. Section 1B: Rh flow cytometry coordinator's report. Rhesus index and antigen density: an analysis of the reproducibility of flow cytometric determination. Transfus Clin Biol 2002;9:33-42.
15. Scott M. Section 1A: Rh serology coordinator's report. Transfus Clin Biol 2002;9:23-9.
16. Ceppellini R, Dunn LC, Turry M. An interaction between alleles at the Rh locus in man which weakens the reactivity of the Rh_o factor (D^u). Proc Natl Acad Sci U S A 1955;41:283-8.
17. Cherif-Zahar B, Raynal V, Gane P, et al. Candidate gene acting as a suppressor of the RH locus in most cases of Rh-deficiency. Nat Genet 1996;12:168-73.
18. Huang C, Cheng GJ, Reid ME, Chen Y. Rhmod syndrome: a family study of the translation-initiator mutation in the Rh50 glycoprotein gene. Am J Hum Genet 1999;64:108-17.
19. Mouro-Chanteloup I, D'Ambrosio AM, Gane P, et al. Cell-surface expression of RhD blood group polypeptide is posttranscriptionally regulated by the RhAG glycoprotein. Blood 2002;100:1038-47.
20. Ogawa K, Orihara T, Hujiwara G, et al. An example of Del producing anti-D. Blood Program 1987;10:76-7.
21. Ueno M, Shinbo K, Furusawa A, et al. A study of Del. Jpn J Blood Transfus Med 1985;31:497-9.
22. Orihara T, Hashimoto A, Hujiwara G, et al. A study of Del [abstract]. Jpn J Blood Transfus Med 1986;32:382.
23. Chen JC, Lin TM, Chen YL, et al. RHD 1227A is an important genetic marker for RhD(el) individuals. Am J Clin Pathol 2004;122:193-8.
24. Westhoff CM. The Rh blood group system in review: a new face for the next decade. Transfusion 2004;44:1663-73.
25. Schmidt PJ, Morrison EG, Shohl J. The antigenicity of the Rho(Du) blood factor. Blood 1962;20:196-202.
26. Flegel WA, Khull SR, Wagner FF. Primary anti-D immunization by weak D type 2 RBCs. Transfusion 2000;40:428-34.
27. Lacey PA, Caskey CR, Werner DJ, Moulds JJ. Fatal hemolytic disease of a newborn due to anti-D in an Rh-positive Du variant mother. Transfusion 1983;23:91-4.
28. Beckers EA, Faas BH, Ligthart P, et al. Characterization of the hybrid RHD gene leading to the partial D category IIIc phenotype. Transfusion 1996;36:567-74.
29. Ostgard P, Fevang F, Kornstad L. Anti-D in a "D positive" mother giving rise to severe haemolytic disease of the newborn: a dilemma in antenatal immunohaematological testing. Acta Paediatr Scand 1986;75:175-8.
30. Cannon M, Pierce R, Taber EB, Schucker J. Fatal hydrops fetalis caused by anti-D in a mother with partial D. Obstet Gynecol 2003;102:1143-5.
31. Hill Z, Vacl J, Kalasova E, et al. Haemolytic disease of newborn due to anti-D antibodies in a Du-positive mother. Vox Sang 1974;27:92-4.
32. White CA, Stedman CM, Frank S. Anti-D antibodies in D- and Du-positive women: a cause of hemolytic disease of the newborn. Am J Obstet Gynecol 1983;145:1069-75.
33. Mollison PL, Engelfriet CP, Contreras M. Blood transfusion in clinical medicine. 10th ed. Oxford: Blackwell Science; 1997.