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Impact of *RHD* genotyping on transfusion practice in Denmark and the U.S. and identification of novel *RHD* alleles

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ABSTRACT

BACKGROUND

Reduced D antigen on RBCs may be due to "partial" D phenotypes associated with loss of epitope(s) and risk for alloimmunization, or "weak" D phenotypes that do not lack major epitopes with absence of clinical complications. Genotyping of samples with weak and discrepant D typing is recommended to guide transfusion and Rh immune globulin prophylaxis. The goal was to compare the impact of *RHD* genotyping on transfusion practice in two centers serving different populations.

MATERIALS AND METHODS

Fifty-seven samples from Denmark and 353 from the U.S. with weak or discrepant D typing were genotyped. RBC typing was by multiple methods and reagents. DNA isolated from WBCs was tested with RBC-Ready Gene D weak or CDE (inno-train) in Denmark, or *RHD* BeadChip (Immucor) in U.S. *RHD* was sequenced for those unresolved.

RESULTS

Of Caucasian samples from Denmark, 90% (n=51) had weak D types 1, 2, or 3; two had other weak D, two partial D, and two new alleles. In diverse ethnic U.S. samples, 44% (n=155) had

weak D types 1, 2, or 3 and 56% (n=198) had other alleles; uncommon weak D (n=13), weak 4.0 (n=62), partial D (n=107), no *RHD* (n=9), and new alleles (n=7).

CONCLUSION

Most samples with weak or variable D typing from Denmark had alleles without risk for anti-D. In U.S. samples, 48% could safely be treated as D+, 18% may require consideration if pregnancy possible, and 34% could potentially benefit from being treated as D–. Black and multiracial ethnicities were overrepresented relative to population.

INTRODUCTION

Individuals whose RBCs type weaker than expected, i.e. serologic weak D, often defined as an agglutination strength of ≤ 2+ with anti-D have always presented a dilemma for transfusion services. A definition of what agglutination strength should be considered weak is not universally relevant for all, as it varies with the reagent and technique utilized.[1] More problematic is the lack of standard practice, as some treat all patients presenting with RBCs with weaker than expected serologic reactivity as D–, some treat as D– only if they are female, and some treat all as D+.[2] The goal of a conservative approach to treat as D– is to avoid sensitization in individuals lacking epitopes of the D antigen who are at risk for clinically significant allo anti-D if exposed to D+ RBCs. However, RBCs lacking D epitopes cannot reliably be distinguished from those with reduced D antigen expression by routine antigen typing. The question of whether to provide patients with "serological weak D phenotypes" with D+ or D– RBCs and platelets, and whether

women who deliver a D+ fetus require Rh immune globulin (RhIg) prophylaxis, cannot be answered by serology alone.

It is recommended in the U.S. that *RHD* genotyping be performed on samples demonstrating weaker than expected reactivity in D typing to determine which patients are at risk of becoming alloimmunized, with the goal of using that information to avoid unnecessary use of RhIg and overuse of D– blood.[2]   Based on observational data primarily from Europe[3-8] and consensus opinion,[9] individuals with weak D types 1, 2, or 3 can safely receive D+ blood products without risk of clinically significant alloimmunization and do not need RhIg prophylaxis. A retrospective look-back by Yazer *et al* in U.S. patients reported that amongst 4,070 recipients with a serological weak D typing, nine (0.2%) were either potentially or likely alloimmunized following D+ RBC or platelet transfusion.[10] *RHD* genotyping was not performed.

Additional studies that determine the frequency of specific *RHD* genes underlying serological weak D phenotypes would be helpful to assess the potential benefit of a genotype approach, recognizing that allele frequencies will differ depending on the population diversity in a specific geographic area. It has been estimated that weak D types 1, 2, and 3 account for approximately 80% of the serologic weak D phenotypes in the Caucasian population in the U.S. and in certain parts of Europe, [11-14] however, the frequency of specific alleles amongst serological weak D individuals in the ethnically diverse U.S. population and other parts of the world vary.[15-17] In this study, blood donors and patients with weak or discrepant D phenotypes in Southern Denmark and in the U.S. were identified, and the underlying *RHD* alleles were determined. The goal was to assess and compare the potential impact of the use of *RHD* genotyping on the D– blood supply, and on the potential for the use of RhIg.

MATERIALS AND METHODS

Denmark: South Danish Transfusion Service, Odense University Hospital (SDTS)

In the setting of a combined transfusion service and regional blood center at a tertiary care hospital in the Region of Southern Denmark, samples were collected from either newly recruited blood donors (n=5,800) or from patients (n=10,000) over a 7-month period. Samples were included in the study if they demonstrated weak (≤2+) or mixed field agglutination with anti-D by AutoVue using BioVue cassettes (Ortho Clinical Diagnostics, Raritan, NJ). Donor samples were also included if they initially tested RhD negative (n=4, AutoVue/BioVue) but had a positive (≥+w) reaction in manual IAT with ID-DiaClon anti-D (ID 09410) in ID-Card Coombs IgG (Bio-Rad, Hercules, CA), or if they demonstrated either weak (≤2+) or mixed field agglutination with anti-D by NEO solid phase analyzer (Immucor, Norcross, GA). All samples underwent further anti-D typing by the following manual methods: Seraclone 232 (Bio-Rad) by tube test, ID-Card Coombs IgG (Diagast, Loos, France), and DiaClon ABO/D + Reverse Grouping ID-gel card (Bio-Rad). All serology was performed according to the manufacturer's instructions, except for testing with DiaClon anti-D, where 25uL reagent was used instead of 50uL as laboratory validation showed no difference in reactivity.

Fifty-seven samples from Danish patients (n=22) or blood donors (n=35) with weak, discrepant, or mixed field D phenotypes were identified amongst the approximately 15,800 routine ABO and RhD types. Data on ethnicity was not available. As the vast majority (approximately 91.4%) of Denmark's population is of Danish origin, the samples were presumed Caucasian [Web resource].

Genomic DNA was isolated from whole blood using Maxwell 16 Blood DNA purification kit (Promega, Denmark). *RHD* genotyping was done using RBC-Ready Gene D weak and those unresolved where tested by RBC-Ready Gene CDE (inno-train, Germany). If no *RHD* variant was detected, amplification and in-house sequencing of all *RHD* exons was

performed (ABI3500, Life Technologies, Denmark) and sequences were analyzed using SeqScape v3.0 (Life Technologies). *RHCE* genotyping was by ID CORE XT[18] (Grifols, Barcelona, Spain).

New York Blood Center (NYBC)

A total of 353 patient samples referred for *RHD* genotyping due to weak or discrepant D typing from local and national hospitals and reference laboratories over a 1-year period were included in the study. Ethnicity was provided for 144. The majority, 52.8%, were White (n=76), 29.9% African American (n=43), 6.9% Multiracial (n=10), 4.9% Latino or Hispanic (n=7), 3.5% Other (n=5), and 2.1% Asian (n=3). RBC samples were tested to confirm reactivity and those found to have novel alleles, or alleles previously identified but without serological characterization, were tested with multiple anti-D including BioClone (Ortho Diagnostics), Seraclone (Bio-Rad), Gamma-clone, Series 4 and 5 (Immucor, Norcross, GA), ALBAclone alpha, delta, and blend anti-D (Quotient, Newtown, PA). ALBAclone advanced partial RhD Kit (Quotient) was tested for some samples.

Genomic DNA was isolated from peripheral blood by standard methods (QIAamp, QIAGEN, Inc., Valencia, CA). *RHD* genotyping was performed with the *RHD* BeadChip array (BioArray/Immucor, Warren, NJ), which targets alleles associated with common partial and weak D phenotypes, and hybrid box assay was performed for *RHD* zygosity.[19] If no *RHD* variant was detected, multiple PCR-RFLP, genomic *RHD* exon-specific sequencing, and/or RhD-cDNA sequencing were performed and analyzed with ClustalX as previously described.[20,21] If no *RHD* gene was detected, *RHCE* BeadChip (Bioarray/Immucor) was performed to investigate *RHCE* variants associated with positive reactivity of some RBCs with monoclonal anti-D reagents in the absence of *RHD*.

RESULTS

Overall, a total of 410 samples, 57 from Denmark and 353 from the U.S., with weak or discrepant RhD typing were studied. The alleles found, designated by country, are graphically illustrated in Figure 1 and summarized in Tables 1 and 2.

All 57 samples (100%) studied from Denmark had altered alleles; 35 from donors and 22 from patients. The frequency of encountering a weak or variable D typing in Danish blood donors, which included those D negative on initial typing and reflexed to indirect antiglobulin testing, was 35/5,800 samples or 0.6%.

In U.S. samples, referred from patients tested by many different methods in local hospitals, 344/353 (97.5%) had altered alleles and the remaining 9 (2.5%) had no *RHD*. The frequency of encountering a weak or variable D typing could not be calculated due to the non-random nature of the samples studied.

Denmark Alleles

Most samples from the Region of Southern Denmark had alleles encoding weak D phenotypes (93%, 53/57) (Table 1A, Figure 1). Forty-five were *RHD*weak D type 1* (79%), five were *weak D type 2* (9%), one *weak D type 3* (2%), and two *weak D type 5* (3,5%). RBCs from samples with weak D types 1 and 2 demonstrated predominantly mixed field agglutination with the BioVue ABO/D cassette, and ≤ 2+ agglutination in DiaClon ABO/D ID-gel card. RBCs from samples with weak D type 3 or type 5 demonstrated either weak 1+ (type 3) or no agglutination in BioVue ABO/D cassette and were non-reactive in DiaClon ABO/D ID-gel card. All 53 samples with weak D alleles demonstrated 3-4+ agglutination with DiaClon anti-D and with DIAGAST anti-D in IgG gel card.

Four of the 57 (7%) samples had other variant alleles. Two (3.5%) had *RHD*DVI type 2* encoding a partial D phenotype. Both RBC samples gave robust agglutination (≥3+) with

DiaClon anti-D in IAT. Two (3.5%) had unreported *RHD* variants; c.431T>C (p.Leu144Pro) and c.1250T>G (p.Phe417Cys) respectively, summarized in Table 2. RBCs from the donor with *RHD*431C* (sample #1) reacted 1-2+ with Seraclone anti-D 232 by tube testing, 3-4+ with ID-DiaClon and DIAGAST anti-D, and were 4+ reactive in BioVue ABO/D cassette and DiaClon ABO/D ID-gel card. RBCs from the patient with *RHD*1250G* (sample #2) demonstrated similar reactivity although mixed field reactivity was observed. Both were *RHCE*Ce/ce*. (Table 2)

U.S. Alleles

Among the 353 referred U.S. samples, alleles encoding weak D represent 47.6% (168/353) (Table 1B, Figure 1). Eighty-eight were *RHD*weak D type 1* (24.9%), 46 were **weak D type 2* (13.0%), and 21 were **weak D type 3* (5.9%). Twelve different uncommon weak D alleles found in 13 (3.7%) included weak D types 6, 15, 40, 42, 45, 51, 57 (n=2), 59, 61, 78, 91, and 119..

A nearly equivalent number, 47.9% (169/353), had alleles encoding other variants including *partial weak D 4.0* (n=62, 17.6%), *DAR* (n=46, 13%), or various DAU alleles associated with partial D (n=26, 7.4%); *DAU2* (n=2), *DAU3* (n=2), *DAU4* (n=7), *DAU5* (n=9), *DAU6* (n=2), *DAU12* (n=1), and compound heterozygotes *DAU3/DAU4* (n=1), *DAU3/DAU5* (n=1), *DAU6/D 4.0* (n=1). Other partial D alleles (n=35, 9.9%) in descending order of prevalence included *DOL* (n=5), *DVII* (n=5), *DFV* (n=4), *DFR* (n=4), *DFW* (n=3), *DVI* (n=2) *DCS1* (n=2), *DLO* (n=2), *D*780A* (n=2), and *DAR4* (n=1), *DBT1* (n=1), *DHMi* (n=1), *DMA* (n=1), *DMB* (n=1), and *RHD*DAR(CE2:V50V-S68N)* (n=1). (Figure 1)

The serologic reactivity of the RBCs associated with the previously reported, *RHD*780A* (c.780C>A, p.His260Gln) and *RHD*DAR(CE2:V50V-S68N)*[22], were not previously described and are summarized in Table 2. RBCs from *RHD*780A* (sample #3) demonstrated very weak to undetectable reactivity at immediate spin (IS) and 2+ at IAT with multiple anti-D (BioClone,

Seraclone, Gamma-clone, Immucor Series 4 and 5). RBCs were not agglutinated with two clones

(LHM 174/102 & 57/17) but demonstrated 2+-3+ reactivity with all 10 other clones in the

ALBAclone partial D kit. RBCs from *RHD*DAR(CE2:V50V-S68N)* (sample #4) reacted weakly

at IS and 2+-3+ at IAT (Immucor Series 4 and 5 and Gamma-clone), but were 2+ at IS with three

anti-D (ALBAclone alpha, delta, and blend). The RBCs did not react with two clones in the

ALBAclone partial D kit (LHM174/102 and 70/45), reacted 1+ with three (LHM57/17, 169/81,

and 59/19), and 2+-3+ with the remaining seven.

Seven of the 353 (2%) had unreported *RHD* variants, summarized in Table 2 (#5-#11).

Two (#5, #6) had the c.1136C>T (p.Thr379Met) change characteristic of DAU alleles with

additional changes, c.787G>T and c.788G>T in exon 5 encoding p.Gly263Leu, designated

RHD*DAU 787_788delinsTT by HGVS nomenclature,[23] and c.761C>T encoding p.Ser254Leu

designated *RHD*DAU(761T).* RBCs from #5 demonstrated microscopic to 1+ agglutination at IS

and 3+- 4+ at IAT (Gamma-clone, Immucor Series 4 and 5, BioClone anti-D), and 2+- 3+ with

anti-D clones in the ALBAclone partial D kit except for LHM174/102 & 57/17. RBCs from # 6

were not detected as D+ positive at IS or IAT with most anti-D (Gamma-clone, Immucor Series 4

and 5, ALBAclone blend and alpha), but were microscopic at IS (ALBAclone delta) and weak

positive at IAT (BioClone). Papain treated RBCs demonstrated 1+^{S} agglutination in IAT with

BioClone anti-D. The two new DAU alleles join 21 alleles to date in the DAU cluster as a major

ancestral African background allele.[24] The DAU allele with c.761C>T is the first DAU family

member to encode a D_{el} like phenotype with failure to detect D antigen by most routine testing.

Similar to the DAU allele with missense c.761C>T, a change at this same position, c.761C>G

was found in Japanese samples but encodes a stop codon p.Ser254Ter.[25] Sample 7 had two novel

polymorphisms, c.520G>A (p.Val174Met) in exon 4 and c.919G>A (p.Gly307Arg) in exon 6.

RBCs demonstrated +w and 2+ agglutination at IS (Bioclone, Gamma-clone or Seraclone, respectively) and 3+ at IAT but the sample was insufficient for additional testing. The c.520G>A and c.919G>A changes have been reported independently on separate alleles as weak D type 33[26] and type 8[11], respectively, but have not been reported on the same allele to our knowledge. Samples 8 and 9 had novel single missense mutations; a c.773T>C (p.Leu258Ser) in exon 5, designated *RHD*773C*, and c.463A>G (p.Met155Val) in exon 3, designated *RHD*463G*. *RHD*733C* was *in trans* to *RHD*DIIIa-CE(4-7)-D* and the RBCs were non-reactive or +w at IS and 2+-3+ at IAT with anti-D (BioClone, Immucor Series 4 and 5, ALBAclone blend, Gamma-clone). RBCs with *RHD*463G* demonstrated 2+-3+ agglutination at IS and 3+ IAT with anti-D (BioClone, Gamma-clone, Immucor Series 4, ALBAclone alpha, delta, and blend). A novel silent change, c.939G>C (p.Pro313Pro) is the last base of exon 6, designated *RHD*939C*, and predicted to cause aberrant splicing of *RHD* transcripts was present in sample #10. The RBCs demonstrated 2+-3+ agglutination with mixed field reactivity at IS with several anti-D (BioClone, Gamma-clone, Seraclone). Recent transfusion and stem cell transplant were ruled out. While the c.939C is novel, a change at this same position, c.939G>A (p.Pro313Pro), was previously reported associated with a weak D serologic phenotype caused by skipping of exon 6.[27] Sample #11 presented with low signal (LS) by DNA testing for exon 2 markers on *RHD* BeadChip, suggesting the presence of a hybrid allele. *RHD*-specific exon 2 amplification and sequencing found a partial conversion with the 5' region of *RHD* replaced by *RHCE* (c.150C, 178C, 201A, 203A) in addition to a previously reported c.254C>G change.[19] RhD-cDNA sequencing confirmed the hybrid *RHD*, designated *RHD*D-ceAG(2)-D*. The RBCs demonstrated 1+-2+ reactivity at IS and 2+-4+ at IAT with multiple anti-D (BioClone, Gamma-clone, Immucor Series 4 and 5, ALBAclone blend, alpha, and delta), and typed G antigen negative. When tested for reactivity with the ALBA partial D typing kit, the RBCs were non-reactive with 3 clones

(LHM174/102, 70/45, and 57/17), +w with 1 (LHM57/17), 1+ with two (LHM169/81 and 59/19), and 2+-3+ with six. *RHCE* present in samples with novel *RHD* are also shown in Table 2.

     RHD genotyping confirmed 9 of the 353 U.S. samples (2.5%) were D negative, with no signal for all exons by *RHD* BeadChip, and confirmed by absence of amplification products by manual testing using primers specific for *RHD* exon 4, 7, and 8. The 9 samples were negative when tested for variants *RHCE*ceCF, *ceHAR, *ceSL, and *ceRT* known to cause positive reactivity with some monoclonal anti-D. The *RH* genotypes of eight were *RHCE*ce/ce* (rr) with one *RHCE*ce/cE* (r''r).

Ethnic Associations

     Ethnicity was not provided for the 57 Denmark samples and were presumed to be Caucasian as all were of Danish origin. The ethnic distribution provided for 144 U.S. samples and the associated alleles are shown in Figure 2. As expected, most of the weak D type 1, 2, and 3 alleles were in samples from Whites. When compared to the U.S. population overall, which at the present time is 60% White, 12% African American, 3% Multiracial, 18% Latino or Hispanic, 3% Other, and 6% Asian,[28] samples from African American and Multiracial groups (30% and 7%, respectively) were overrepresented among samples with weak or discordant D typing referred for genotyping.

Females and Pregnancy Considerations

     Among samples from Denmark and the U.S. for which age and gender were provided, 40% from Denmark and 67% from U.S. were females under age 50. (Table 1C) Of samples from both countries with weak D types 1, 2, and 3, 119 (62%) were females of child-bearing age. Among 62 with weak D 4.0, 35 (56%) were females under age 50, as were 77 of 118 (65%) with partial *RHD*, no *RHD*, or new alleles.

DISCUSSION

Patient and donor samples from the Region of Southern Denmark and the U.S. encountered over approximately 1 year (7-12 months, respectively), with weaker than expected or discrepant D serologic typing were genotyped for *RHD* to determine if the patient could be treated as D+ or would be better treated as D–, and to begin to estimate the overall impact of *RHD* genotyping on clinical practice. Individuals with weak D types 1, 2, and 3 can safely be treated as D+ and are more frequent in the Caucasian population.[5,11,12,29,30] In addition, although anti-D has occasionally been reported, no clinically significant HDFN or transfusion reactions have been reported in individuals with other uncommon weak D types. Individuals with partial D phenotypes, who most agree would be better treated as D– to avoid anti-D if there is potential for a future pregnancy or possibly chronic transfusion, are also associated with specific ethnic groups. For example, the partial DVI phenotype is primarily found in European-Caucasians[31,32], and partial DIIIa, DAR, various DAU, and DOL phenotypes are predominantly found in Black and Hispanics.[17,24,33-36] However, the self-declared or assumed ethnicity of a patient is not a basis for determining appropriate clinical therapy.

In this study of the Region of Southern Danish population, the frequency of encountering a serologic weak or variable D typing was 0.6% (35/5,800). Most, 93% were due to inheritance of a weak D allele without risk for anti-D, consistent with previous studies from Europe,[11,12] and most certainly due to the lack of diversity in the population tested. In the Danish study, it was observed that RBCs from samples with weak D types 1 and 2 often demonstrated mixed field agglutination when tested with the BioVue ABO/D cassette. Mixed field agglutination associated with weak D type 1 and 2 has also been occasionally noted in tube testing with some reagents in the U.S. (our unpublished observations). Two females of child-bearing age had partial DVI phenotypes with serious risk for clinically significant alloimmunization [37,38] and recommended to

be treated as D−. Both individuals, one male and a female under the age of 50, with novel alleles would be treated conservatively as D− in the Danish system, although if D− blood was in limited supply the male would receive D+. Overall, approximately 93% of individuals with weak or variable D typing in Demark are safely treated as D+ for transfusion or pregnancy and 7% would be treated as D−.

In the ethnically diverse U.S. population, the frequency of encountering a serologic weak or variable D typing could not be calculated in this study as all samples were referred, although previous studies suggest the frequency is 1-4%,[39-41] depending on the method and the population tested. RH genetic diversity in different ethnic groups[42] requires choosing appropriate targets for accurate *RHD* genotyping, as has been discussed for cell free fetal D determination in the U.S.[43]

In the U.S. cohort, nearly half, 47.6%, had weak D alleles. The majority (43.9%) were weak D type 1, 2, or 3 and should be treated as D+. For less common weak D (3.7%), the clinical decision on how to treat should consider the blood supply and transfusion needs as well as the age and gender of the individual and, importantly, consider that no HDFN or hemolytic transfusion reactions have been reported to date associated with a weak D allele. The remainder, 52.4%, had alleles encoding partial D phenotypes (n=107, 30.3%) or D− phenotypes (n=9, 2.5%) with risk for clinically significant anti-D, new alleles (n=7, 2%) with unknown risk for anti-D, and weak partial D 4.0 (n=62, 17.6%).

Anti-D in patients with weak partial D 4.0 has been the subject of much discussion.[44,45] *RHD*weak partial D 4.0* has an allele frequency of 0.02 to 0.049 in African Americans,[46,47] and although anti-D has not been reported to be associated with weak D 4.0 in Europe, where the allele is uncommon, or in Tunisia where the allele is frequent,[48] anti-D was identified in 3 of 16 children with SCD. Thirty-eight individuals, 13 of them pregnant women, reported by two large

reference laboratories had anti-D,[44] hence this allele was called "partial weak D" by ISBT. The serologic reactivity of the antibody made by individuals with this allele can be confusing as the anti-D can have both allo and auto characteristics. No HDFN, anemia, or hemolytic transfusion reactions have been reported.[44] In the absence of reports of clinical sequelae, the decision to treat as D+ or D– should consider the age and sex of the patient, as well as the D– blood supply, and for females, the cost of following a pregnancy that will be seen by most clinicians as having the potential for risk. However, based on the allele frequency, supporting every patient with weak D 4.0 with D– blood to prevent any potential for anti-D production would be a potential burden for the D– blood supply.

The overall prevalence of new alleles did not vary significantly between Denmark (3.5%) and the U.S. (2%). In the absence of clinical information regarding risk for anti-D, testing of the RBCs with epitope-specific anti-D can give potential insight. The absence of epitopes revealed by the lack of reactivity with some clones (samples 3, 4, 5, 11; Table 2) suggest these alleles encode partial D phenotypes with potential risk for D alloimmunization. The type of mutation also gives insight, for example, the *RHD*939C* change occurs in an exon splice site and is predicted to result in a reduced level of RhD transcripts responsible for the weak D typing. In the absence of an amino acid change, this allele would not be predicted to be associated with anti-D. Overall, approximately 48% of individuals with weak or variable D typing in the U.S. can safely be treated as D+ for transfusion or pregnancy, 18% may require consideration if future pregnancy is possible, and 34% are predicted to benefit from being treated as D–.

The decision to treat as D+ or D– should consider patient age, gender, history of antibody production or responder status, future transfusion needs, and the blood supply in addition to the specific allele present. Amongst the individuals with weak D types 1, 2, or 3, sixty-two percent were females of child-bearing age who could avoid unnecessary RhIg injection in pregnancy, and

the unnecessary use of D– blood would be avoided for all by *RHD* genotyping. Among those

with other alleles, or absence of *RHD*, 64% were females under age 50 who could potentially

benefit from being treated as D–for transfusion and pregnancy. Considerations on how to treat

males and older females should be based on diagnosis and avoid challenging the D– blood

supply. The financial implications to the health care system remains unclear with *RHD*

genotyping strategies that include testing of males and older females.

Weak D in Denmark has a high probability of being weak D types 1, 2, and 3 and 93%

could be safely transfused with D+ products and females do not require RhIg. In the US, the

probability of having an allele that is not weak D types 1, 2, or 3 was 56%. Although the focus is

often on serologic weak D phenotypes associated with weak D types 1, 2, and 3 common in

Caucasians to avoid unnecessary use of RhIg and D– blood, African Americans who more often

have partial D phenotypes are over-represented in U.S. samples presenting with weaker than

expected or discordant D typing. Many of the multiracial individuals have partial D phenotypes

which emphasizes a benefit of *RHD* genotyping in ethnically diverse populations to provide

appropriate transfusion and RhIg administration decisions. In summary, *RHD* genotyping if

made standard of practice for determining the D status of all donors and patients, would improve

patient care by guiding appropriate therapy and would support the use and allocation of D–

resources.

Legends

Figure 1. RHD alleles in samples with weak or discordant D phenotypes. Number of

samples of each type of allele or allele combination are indicated. Grey bars indicate

Denmark and black bars U.S. samples. The sample axis is scaled in increments from 1 to

5 and 10 to 90 to allow visualization of the relative numbers of alleles with lower

prevalence.

Figure 2. Specific RHD alleles present in 144 U.S. patients and the associated ethnic

ancestry represented by open, filled, patterned or hatched bars. Weak D alleles are

predominant in individuals identifying as White and clinically important partial D (DAR

and Other partial D) are found in all ethnic groups. The graphical representation for each

group is shown in the legend.

Table 1. Number of samples by RhD phenotypes or with new alleles and % of total. A). Denmark, B). U.S. and C). according to gender and age, i.e. females ≤ 50 years of age or males and females >50 for all 57 samples from Denmark and for 329 of 353 from U.S.

A. Denmark

	Weak D				Partial D	New *RHD*
	1	2	3	5	DVI	
# Samples	45	5	1	2	2	2
Percent	89.5%			3.5%	3.5%	3.5%
	93%				7%	

B. U.S.

	Weak D				Weak/partial 4.0	Partial D			No *RHD*	New *RHD*
	1	2	3	Other	4.0	DAR	DAU	Other		
# Samples	88	46	21	13	62	46	26	35	9	7
Percent	43.9%			3.7%	17.6%	30.3%			2.5%	2.0%
	47.6%				47.9%				4.5%	

C. Gender and age.

Phenotypes	Denmark (n=57)		U.S. (n=329)		Total
	Females ≤50	Males & females >50	Females ≤50	Males & Females >50	
weak D types 1, 2, and 3	18	33	101	39	191
Other weak D	2	0	8	5	15
weak D 4.0	N/A	N/A	35	27	62
Partial D	2	0	68	32	102
No RhD	N/A	N/A	4	3	7
New RhD	1	1	5	2	9

Total	23 40%	34 60%	221 67%	108 33%	

Table 2. Nine new alleles detected in this study (samples 1, 2, 5-11) and two alleles (samples 3, 4[22]) for which typing results had not been described. The nucleotide and amino acid changes, GenBank accession number, rs SNP reference number, serologic results testing with multiple anti-D and ALBAclone advanced partial D kit for samples with sufficient RBCs, and *RHCE* genotype are shown.

Center	Sample	Allele designation	Nucleotide change	Amino acid change	GenBank#	rs#	Agglutination with anti-D reagents		Agglutination with ALBAclone partial D kit	*RHCE**
							IS	**IAT**		
Denmark	1	*RHD*431C*	c.431T>C	p.Leu144Pro	MK542002	rs775007210	1+	3+ - 4+	NT	*Ce/ce*
	2	*RHD*1250G*	c.1250T>G	p.Phe417Cys	MK542003	NF	1+s	3+ - 4+mf	NT	*Ce/ce*
NYBC	3	*RHD*780A*£	c.780C>A	p.His260Gln	KU363612	rs1170243924	0 – 1+	2+	0 with LHM 174/102 & 57/17	*ce/ce48C*
	4	*RHD*DAR(CE2:V50V -S68N)*£	c.150T>C c.178A>C c.201G>A c.203G>A c.602C>G c.667T>G c.957 G>A c.1025T>C	silent p.Ile60Leu p.Ser68Asn p.Thr201Arg p.Phe223Val silent p.Ile342Thr	HG423860	rs1132758 rs1053341 rs41302032 rs62621068 rs1053355 rs1053356 rs146292192 rs138235491	+w - 2+	2+ - 3+	0 with LHM174/102 & 70/45	*ce/ceAR*
	5	*RHD*DAU (787_788delinsTT)*	c.787_788delinsTT c.1136C>T	p.Gly263Leu p.Thr379Met	KY680215	rs1264388315 rs61740966	mi+-1+	3+ - 4+	0 with LHM 174/102 & 57/17	*ce/ce*
	6	*RHD*DAU (761T)*	c.761C>T c.1136C>T	p.Ser254Leu p.Thr379Met	KY680216	NF rs61740966	0 - mi+	0 – w+#	NT	*ce48C/ce*
	7	*RHD*520A, 919A*	c.520G>A c.919G>A	p.Val174Met p.Gly307Arg	KY926704	rs147421281 rs762881746	+vw - 2+	2+ - 3+	NT	*Ce/ce*
	8	*RHD*773C*	c.773T>C	p.Leu258Ser	KY652758	NF	0 - +w	2+ - 3+	NT	*cE/ceS*
	9	*RHD*463G*	c.463A>G	p.Met155Val	KY680214	NF	2+ - 3+	3+	NT	*Ce/ce*

10	*RHD*939C*	c.939G>C	p.Pro313Pro	KY652759	rs200162404	2+^{mf} - 3+^{mf}	NT	NT	*Ce/ce*
11	*RHD*D-ceAG(2)-D*	c.150T>C c.178A>C c.201G>A c.203G>A c.254C>G c.744C>T	silent p.Ile60Leu silent p.Ser68Asn p.Ala85Gly silent	KY926709	rs1132758 rs1053341 rs41302032 rs62621068 rs139501061 rs1053362	1+ - 2+	2+ - 3+	0 with LHM174/102, 70/45, & 57/17	*ce/ce733G*

IS = immediate spin, IAT= indirect antiglobulin test, vw=very weak, mi=microscopic, mf=mixed field, ^{S}=strong, NT= not tested, NF=not found, $^{£}$=previously reported, $^{#}$Enzyme treated cells 1+^{s}

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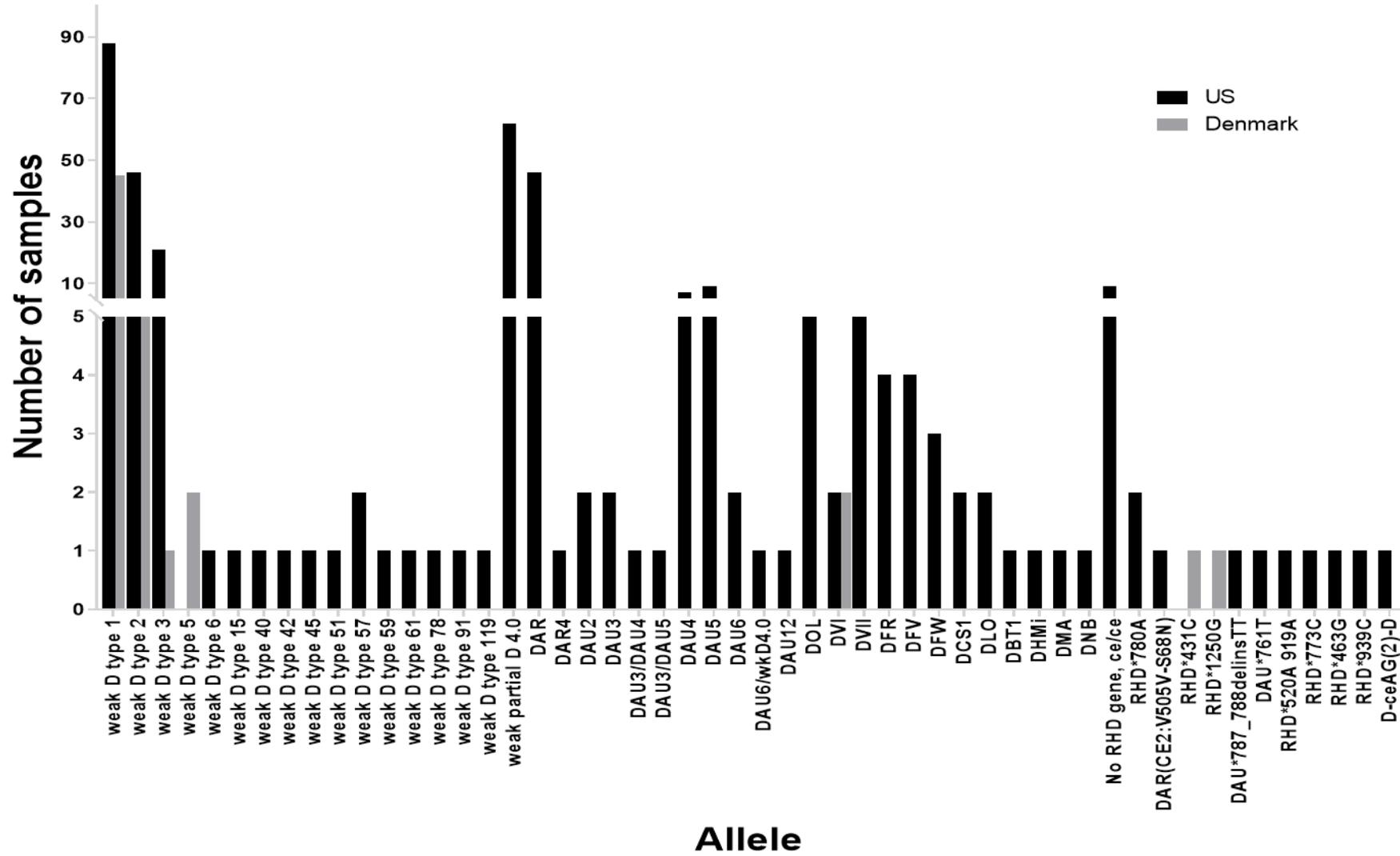
Figure 1

Figure 2