Molecular basis of D variants in Chinese persons

Lixing Yan,* Junjie Wu,* Faming Zhu, Xiaozhen Hong, and Xianguo Xu

BACKGROUND: Most studies of the molecular basis of D variants have been conducted in Caucasian and African populations. There are limited data on the molecular basis for D variants in Chinese populations.

STUDY DESIGN AND METHODS: With a blended monoclonal anti-D and a sequential slide and tube typing protocol, red blood cells from greater than 99 percent of Han Chinese were tested for the D antigen. Samples that agglutinated weakly by tube method or that reacted only by indirect antiglobulin test (IAT) were classified as D variants. The D variant was tested by an \textit{RHD} polymerase chain reaction with sequence specific primers and by gene sequencing to distinguish and characterize weak D and partial D alleles.

RESULTS: Of 305,572 samples from individual donations (305,475 [99.97\%] were ethnic Han and 97 [0.032\%] were ethnic minorities), 304,134 (99.53\%) typed as D\textsuperscript{+}. Five (0.0016\%) typed as D variants (weak agglutination by tube). By IAT an additional 32 (0.0105\%) typed as D variants and 1401 (0.46\%) typed as D\textsuperscript{−}. Weak D type 15 and \textit{RHD(K409K)} alleles represented 72.7 percent of all weak D phenotypes. All partial D phenotypes were DVI Type 3 or DV. Three new weak D alleles carrying 594A>T and 602C>G (weak D Type 51), 92T>C (weak D type 52), and 740T>G (weak D type 53) mutations, respectively, were identified.

CONCLUSION: There are significant differences in the frequencies and molecular characteristics of D variants among indigenous Chinese populations, compared to Caucasian and African populations, which must be considered when developing clinical practices related to D variant blood donors, transfusion recipients, or obstetrical patients.

STUDIES of D variant phenotypes in Caucasian and African populations have provided considerable information about their frequency and molecular characteristics.\textsuperscript{1–12} Relatively few studies of \textit{RHD} alleles have been conducted among Asians and, in particular, indigenous Chinese.\textsuperscript{13–21} Except for certain focused analysis of Rh phenotypes or haplotypes in specific geographic or ethnic populations, molecular research of the Rh blood group system in indigenous Chinese has been limited to gene structure analysis and genotyping by polymerase chain reaction with sequence-specific primers (PCR-SSP).\textsuperscript{22,23} To our knowledge, only 10 examples of weak D and one partial D phenotype have been defined in indigenous Chinese by molecular methods.\textsuperscript{13–15} Despite the relatively low frequency of D\textsuperscript{−} phenotypes among Chinese, nearly 30 percent have grossly intact \textit{RHD} genes and express the DEL phenotype.\textsuperscript{13,16,17,24,25} We consider the relatively high percentage of weakly expressed D antigens in Chinese to suggest the likelihood of polymorphism \textit{RHD} genes and D variant phenotypes in this population. The following report describes the results of our studies of D variant phenotypes in an indigenous Chinese population.

MATERIALS AND METHODS

Study population

All donors resided in Zhejiang Province, which is located in the southern region of the Yangtze River delta in south-

ABBREVIATION: PCR-SSP = polymerase chain reaction with sequence-specific primers.

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eastern China. Of Zhejiang Province’s 46,470,000 inhabitants, an estimated 99.1 percent are ethnic Han. The remaining 400,000 inhabitants are distributed among 53 ethnic minorities, including 200,000 ethnic She.

Serologic studies
During a 4-year study period, we typed all blood donors at the Blood Center of Zhejiang Province for the D antigen by a sequential procedure with slide and tube methods and a monoclonal-monoclonal blended anti-D reagent (Novacline, Dominion Biologicals Inc., Dartmouth, Nova Scotia, Canada). This reagent contains immunoglobulin M (IgM) from cell line DBL D175-2 and IgG from cell line DBL 415 1E4. For the slide test, we added two drops of whole blood from a segment of blood bag (approximately a 40%-50% suspension) to a slide and mixed it with one drop of anti-D over a 1.5 x 1.5-cm area with a clean wooden applicator. We tilted the slides gently and continuously and observed for agglutination within 2 minutes, according to the method described in the AABB Technical Manual. We classified samples that agglutinated as D+. We retested all samples with negative or equivocal agglutination by tube method. For the tube method, red blood cells (RBCs) were washed three times with 0.9 percent sodium chloride and prepared as a 2 to 5 percent suspension. We used a three-donor pool of D+ RBCs as a positive control and C–c-5 percent suspension. We used a three-donor pool of D+ RBCs with 0.9 percent sodium chloride and prepared as a positive control. We washed RBCs three times continuously and observed for agglutination within 5 minutes; 35 cycles at 94°C for 30 seconds, at 64°C for 30 seconds (67°C for exons 4, 6, 8, 9, and 10; 60°C for exon 5), and at 72°C for 1 minute; and final extension at 72°C for 10 minutes.

Sequencing analysis
PCR products were purified with PCR purification kit (QiAquick, Qiagen GmbH, Düsseldorf, Germany). Nucleotide sequencing was performed with a DNA sequencing kit (BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase, FS, Applied Biosystems, Foster City, CA) and sequenced (ABI 377, Applied Biosystems). Based on the amplification patterns, the mutation types, and their predicted membrane localizations, we categorized samples as putative weak D or putative partial D according to the definitions of Wagner and coworkers.

D zygosity test
We performed D zygosity tests on all samples that were categorized as D variants, with the PCR-SSP method of Perco and colleagues, which specifically amplifies a 2778-bp fragment of the hybrid Rhesus box.

RESULTS
Study population
During the 4-year study, there were 363,343 blood donations at the blood center, including 57,771 repeat donations, which were excluded from the study. Among a total of 305,572 individual blood donor samples, 305,475 (99.97%) were from ethnic Han and 97 (0.032%) were from ethnic minorities. The samples from ethnic minorities consisted of 38 from She, 17 from Man, 9 from Miao, and 33 from 14 other ethnic minorities.

Serologic studies
Of the total 305,572 individual samples, 304,134 (99.53%) typed as D+ by the slide method. The remaining 1438 samples that did not agglutinate macroscopically by slide method were retested by tube method. Of these, 5 (0.0016%) demonstrated weak agglutination (≤2+) by tube method compared to controls (4+) and were categorized as D variants. Among the remaining 1433 samples that tested negative by tube method, 32 (0.010%) tested D+ by IAT and were categorized as D variants. The
<table>
<thead>
<tr>
<th>Primer denotation*</th>
<th>Previous name†</th>
<th>Sequence 5′ to 3′‡</th>
<th>Specificity</th>
<th>GenBank accession number</th>
<th>Genomic region</th>
<th>Position‡</th>
<th>Product size (bp)</th>
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<tr>
<td>E1-s(=E1-seq) Re01</td>
<td></td>
<td>TCCATAGAGGGGCGACCCAA</td>
<td>D</td>
<td>AJ252314</td>
<td>Promoter</td>
<td>−152 to −132</td>
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<td>E1-a Ds1a</td>
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<td>GCTATTGCTCCTGAGACCCTT</td>
<td>D</td>
<td>Z97363</td>
<td>Intron1</td>
<td>+40 to +18</td>
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<tr>
<td>E2-s Ds2s</td>
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<td>TGAGAAATCTCAGCTCTCAAT</td>
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<td>U66341</td>
<td>Intron1</td>
<td>−1060 to −1037</td>
<td>330</td>
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<td>E2-a Ds2a</td>
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<td>CTGGATCTTGGTCTGACTACG</td>
<td>D</td>
<td>U66341, U66340</td>
<td>Intron2</td>
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<td>219</td>
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<td>E3-s rb20d</td>
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<td>GTGGTCCTGCTGCTCTCTCT</td>
<td>D</td>
<td>AB035190</td>
<td>Intron3</td>
<td>−29 to −8</td>
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<tr>
<td>E3-a Ds3a</td>
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<td>CTGGATCTGCTGCTCTCTCT</td>
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<td>AB035192, AB035191</td>
<td>Intron3</td>
<td>+39 to +19</td>
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<td>Intron3</td>
<td>+28 to +11</td>
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<td>GGCAACCCTACTGCTCTTCAG</td>
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<td>U77079, U77078</td>
<td>Intron3</td>
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<td>984</td>
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<tr>
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<td>Intron4</td>
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<td>Y10605</td>
<td>Intron4</td>
<td>+82 to +64</td>
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<tr>
<td>E5-s rb11</td>
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<td>TACCTTTGAATTAGCACTTAC</td>
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<tr>
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<td>TTATGGCTACTTGGCTGAC</td>
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<td>Z97334, AB035197</td>
<td>Intron5</td>
<td>+1024 to +1006</td>
<td>770</td>
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<tr>
<td>E5-seq rb24</td>
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<td>AGACCTTGGAGGAGGTTG</td>
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<td>Y10605, Y10604</td>
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<td>E5-s§</td>
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<td>D</td>
<td>AJ299023</td>
<td>Exon4</td>
<td>−502 to −484</td>
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<td>GCTGACTCTGCATCAGTG</td>
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<td>Intron5</td>
<td>+315 to +297</td>
<td>274</td>
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<tr>
<td>E6-s(=E6-seq) Ds6a</td>
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<td>CAGGGTGCTGGCTGCAAC</td>
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<td>Z97334, Z97333</td>
<td>Intron6</td>
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<tr>
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<td>CAGGGTGCTGGCTGCAAC</td>
<td>D</td>
<td>Z97334</td>
<td>Intron6</td>
<td>+41 to +21</td>
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<td>E7-s re621</td>
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<td>D</td>
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<td>Intron6</td>
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<td>D</td>
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<td>Intron6</td>
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<td>E8-s Ds8s</td>
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<td>AB035196</td>
<td>Intron8</td>
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<td>E9-s(=E9-seq) Ds9a</td>
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<td>E9-a Ds9a</td>
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<td>GTGAGAGGCTGCTGAGATAC</td>
<td>D</td>
<td>AB035196</td>
<td>Intron8</td>
<td>+294 to +275</td>
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<tr>
<td>E10-s re91</td>
<td></td>
<td>CAGAGATCAGCAGAAAATACGT</td>
<td>D</td>
<td>AB035185, AB035184</td>
<td>Intron9</td>
<td>−67 to −45</td>
<td>381</td>
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<tr>
<td>E10-a rr4</td>
<td></td>
<td>AGCTTACGGATGACACACCA</td>
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<td>X63097</td>
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<td>AGCTTACGGATGACACACCA</td>
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<td>3UTR</td>
<td>+261 to +234</td>
<td></td>
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</tbody>
</table>

* s = sense primer; a = antisense primer; seq = sequencing primer.
† Part of sequence in bold type denoted they were appended on the basis of original primers.
‡ The positions of the synthetic oligonucleotides are indicated relative to their distances from the first nucleotide position of the start codon ATG for all primers in the promoter and in the exons or relative to their adjacent exon-intron boundaries for all other primers.
§ A second set of primers for amplification and sequencing RHD exon 5 was used when the original primers failed to amplify exon 5 or gave weak amplification results.
remained 1401 (0.46%) samples were typed as D– by IAT. These 1401 samples were not tested for the DEL phenotype by adsorption-elution. There was insufficient volume for further testing of 5 IAT-only positive samples, leaving a net of 32 samples (5 D weakly positive and 27 IAT positive) for molecular studies. All 32 samples were from ethnic Han (13 women and 19 men). No blood group alloantibodies were detected in the plasma of these samples.

Molecular characterization of D variants
Among the 32 D variant samples that were available for further study by PCR-SSP, we detected 11 distinct alleles. We categorized 7 of these alleles as weak D and 4 as partial D alleles (Table 2).

Weak D alleles
One sample carried 594A>T and 602C>G missense mutations in exon 4 that led to 198Lys>Asn and 201Thr>Arg mutations located in the third intracellular loop. Because both mutations lay in intracellular loop of D protein, RHHD (594T, 602G) was proposed as a probable weak D allele and named as weak D type 51.\(^\text{29}\) We detected a second new probable weak D allele, named as weak D Type 52,\(^\text{29}\) associated with a 92T>C mutation in exon 1, resulting in a 31Phe>Ser substitution in the first D transmembrane segment. The third new probable weak D allele, named as weak D Type 53,\(^\text{29}\) carried a 740T>G mutation in exon 5, which led to a 247Val>Gly substitution in the eighth D transmembrane segment. Also, we detected 11 examples of weak D type 15,\(^\text{11}\) 2 examples of weak D Type 33,\(^\text{14}\) and 1 example of weak D Type 24.\(^\text{19}\) Finally, we detected 5 examples of RHHD(K409K),\(^\text{13,30}\) among which 1 IAT-only D+ sample and one D-weakly positive sample carried a heterozygous RHHD(K409K) allele with CD\(^{409K}\)e/CDe and CD\(^{409K}\)e/cDDe haplotype (determined by family studies, data not shown), and the other 3 IAT-only D+ samples carried CDe haplotype. Among the 32 D variant samples in our study, 22 were weak D phenotypes representing 7 distinct alleles. Weak D Type 15 and RHHD(K409K) alleles represented 72.7 percent of all weak D phenotypes.

Partial D alleles
One allele with 667T>G and 697G>C mutations on exon 5 was typical of DV Type 1,\(^\text{4,20,29}\) another allele associated with 667T>G, 676G>C, 697G>C, 712G>A, 733G>C, 744C>T, 787G>A, and 800A>T mutations was classified as DV Type 2.\(^\text{4,15,29}\) A third allele representing 667T>G, 676G>C, and 712G>A, 733G>C, and 744C>T mutations was typical of DV Type 8.\(^\text{21,29}\) The remaining 7 samples gave negative amplifications of RHD exons 3 through 6 and were categorized as DVI Type 3, according to the exon amplification pattern proposed by Wagner and coworkers.\(^\text{2}\) Among the 32 D variant samples, 10 were partial D phenotypes representing 4 distinct alleles. DVI Type 3 and DV represented all partial D phenotypes, and the DVI Type 3 allele is the most important partial D allele.

D zygosity test
Three D variants tested negative for hybrid Rhesus box (one carried a homozygous RHHD(K409K) allele, the other two carried a heterozygous RHHD(K409K) allele), whereas the other 29 D variant samples tested positive.

DISCUSSION
The results of our study confirm the considerable polymorphism of RHHD among indigenous Chinese and identify three new alleles associated with the weak D phenotype. Although two of three new alleles were detected from D-weakly positive samples, which probably had been excluded from the investigation by the previous studies,\(^\text{13-15}\) all other eight alleles had been observed in six previously conducted studies focusing on Chinese and Japanese populations.\(^\text{1-11}\) Weak D Type 15 and DVI Type 3 constituted 35 and 22 percent of the 32 D variants detected, which suggests they are the major weak D and partial D alleles in Chinese. More D variant RBC samples must be collected and analyzed before we can predict the frequency of D variants in Chinese. Also, in the absence of standardized typing reagents and serologic methods for detecting D– and D variant phenotypes, any comparison of gene or phenotype frequencies between various populations and/or published studies must be made with considerable caution. Nevertheless, we believe that our results, together with others,\(^\text{13-25}\) indicate that the prevalence and molecular basis of D variants in indigenous Chinese are significantly different from those in European and African populations.\(^\text{1-11}\)

With sequential slide and tube methods, as well as a potent blended monoclonal anti-D typing reagent, we detected 5 weakly reactive (i.e., D variant) samples among 305,572 individual blood donor samples. The relatively low percentage of D variant samples detected (5/305,572) suggests that we classified some samples as D+ that would have been classified as D variants by a less sensitive typing protocol. In contrast, the presence of 27 IAT-only D variant samples among the 1433 samples that were initially categorized as D– by the tube method indicates that the IAT remains an important part of complete D typing in Chinese populations. This latter observation is supported by the reports that even the weakest D variant, DEL, can cause alloimmunization if transfused to a D– recipient\(^\text{31}\) and that D antigen of weak Ds are immunogenic,\(^\text{32}\) although to a major extent whether or not an alloanti-D is induced depends on the immunologic condition of the transfusion recipient.\(^\text{32,33}\)
### TABLE 2. Weak D and partial D alleles in indigenous Chinese

<table>
<thead>
<tr>
<th>Allele name*</th>
<th>Agglutination in tube test†</th>
<th>Nucleotide change</th>
<th>Amino acid substitution</th>
<th>Membrane localization‡</th>
<th>Haplotype</th>
<th>Population study</th>
</tr>
</thead>
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<tr>
<td>Weak D type 15</td>
<td>0</td>
<td>845G&gt;A</td>
<td>G282D</td>
<td>TM</td>
<td>cDE</td>
<td>11 (34.4)</td>
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<td>Weak D type 24</td>
<td>0</td>
<td>1013T&gt;C</td>
<td>L338P</td>
<td>TM</td>
<td>cDE</td>
<td>1 (3.1)</td>
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<tr>
<td>Weak D type 33</td>
<td>2+</td>
<td>520G&gt;A</td>
<td>V174M</td>
<td>TM</td>
<td>CDc</td>
<td>2 (6.3)</td>
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<tr>
<td>Weak D type 51</td>
<td>2+</td>
<td>594A&gt;T, 602C&gt;G</td>
<td>K198N, T201R</td>
<td>IC</td>
<td>CDc</td>
<td>1 (3.1)</td>
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<td>Weak D type 52</td>
<td>1+</td>
<td>92T&gt;C</td>
<td>F31S</td>
<td>TM</td>
<td>CDc</td>
<td>1 (3.1)</td>
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<td>Weak D type 53</td>
<td>0</td>
<td>740T&gt;G</td>
<td>V247G</td>
<td>TM</td>
<td>CDc</td>
<td>1 (3.1)</td>
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<td>RHD(K409K)§</td>
<td>0/(2+)</td>
<td>127G&gt;A</td>
<td>K409K</td>
<td>/</td>
<td>CDc, CDc, CDc</td>
<td>5 (15.6)</td>
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<td>DV Type 110,24</td>
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<td>667T&gt;G, 697G&gt;C</td>
<td>F223V, E233Q</td>
<td>TM, EF</td>
<td>CDc</td>
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<td>F223V-K267M</td>
<td>IC, TM, EF</td>
<td>CDc</td>
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<td>667T&gt;G, 676G&gt;C, 697G&gt;C, 712G&gt;A, 733G&gt;C, 744C&gt;T, 787G&gt;A, and 800A&gt;T</td>
<td>F223V-V245L</td>
<td>TM, EF</td>
<td>CDc</td>
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<td>DVI Type 3</td>
<td>0</td>
<td>RHD-CE(3-6)-D</td>
<td>/</td>
<td>IC, TM, EF</td>
<td>CDc</td>
<td>7 (21.9)</td>
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</table>

* The new RHD alleles had been deposited in GenBank under the Accession Number DQ088170 (weak D Type 51), DQ121434 (weak D Type 52), and DQ 121435 (weak D Type 53).
† RBCs that did not agglutinate in tube test were denoted as "0" here; one proband with a heterozygous RHD(K409K) allele (CDc) gave a 2+ agglutination strength.
‡ Three types of membrane localization of amino acid substitution, IC (intracellular), EF (exofacial), and TM (transmembraneous) were predicted according to the model for orientation of the RhD protein proposed by Wagner and coworkers.11
§ Among five RHD(K409K) alleles, three carry a CDc haplotype, one carries CDc, and one carries CDc.
¶ DVI Type 3 had RHD exons 3 through 6 replaced by corresponding part of RHCE gene.2
Interestingly, RHD(K409K), usually detected among DEL phenotypes by adsorption-elution and representing approximately 30 percent of apparent D– in Chinese persons, was found in five D variants in our study, of which one (CDK409Ke/cDE) demonstrated 2+ agglutination by the tube test and four (one CDK409Ke/cDe, three CDK409Ke) were detected by IAT.

Heterozygous RHD(K409K) allele carriers usually type as normal D+ phenotypes. The D variant with heterozygous RHD(K409K) allele in our study may carry another grossly intact allele in trans, either as a nonfunctional allele, such as RHD 270A, or as a weakly expressed allele such as RHD (IVS5-38del4). If such a mutation is located in an intron, it might not be detected by the RHD gene sequencing method used in our study. More likely, we would expect RHD(K409K) to weaken the expression of a normal RHD gene, since Chinese DEL phenotypes have been observed to carry a heterozygous RHD(K409K) allele.

In previous studies, Shao and colleagues found RHD(K409K) in DEL and weak D phenotypes in indigenous Chinese. Wagner and coworkers found weak D Type 11 allele in DEL and weak D phenotype in European persons. Our finding of RHD(K409K) among D variant samples suggests that the D antigen carrying RHD(K409K) is not necessarily as weak as that of the DEL phenotype and therefore one allele might express more than one phenotype.

Our results of D zygosity testing indicate that 29 D variants carried an RHD gene deletion haplotype, in addition to a D variant allele. Among the 3 D variants with negative hybrid Rhesus box, two D variants with a heterozygous RHD(K409K) allele undoubtedly carried two RHD genes and excluded the possibility of false-negative D zygosity test result. The molecular mechanism for the remaining D variant sample is unknown. It may have reflected the combination of two RHD(K409K) alleles, a previously observed phenomenon among DEL phenotype. That possibility remains to be resolved by family studies. Considering the mutations recently reported to be associated with Rhesus box, it is possible that a mutation on Rhesus box resulted in false negative (or positive) results in our study. Therefore, it would be beneficial to test for hybrid Rhesus box with a complementary method or to sequence the underlying variant region in the next stage.

Together with the findings of Lan and coworkers, Shao and coworkers, Lin and coworkers, and others, our results contribute to increasing knowledge of the differences in the molecular genetics and expression of the D antigen among Asian, European, and African populations. It also confirms that “weak D” is not a single D+, but weak Ds, a heterogeneous group of D peptides, frequencies of which depends on the typing methods, serologic cutoff, and affinity of anti-D. Although these differences are of interest from the perspective of the evolution and development of the Rh blood group system, they also have practical implications for transfusion services serving Chinese populations, because the population frequencies of weak D types and the qualitative changes of D antigen are two of three major factors contributing to an improved transfusion strategy as proposed by Wagner and coworkers.

Probably, the most pertinent difference relates to the disparate frequencies of RHD, itself, among these populations. Given the very high frequency of D+ in Taiwanese persons (>99.6%), one hospital in Taipei discontinued testing donors and patients for D in 1988. The practice of routine testing transfusion recipients and blood donors for D had been introduced in the Taipei hospital, as elsewhere, based on data from Caucasian populations where the frequency of the D+ is approximately 85 percent. Discontinuing routine D typing in the Taiwanese hospital did not result in incremental incidence of anti-D, suggesting how certain Rh-related transfusion practices may be optimized for Asian populations by relying on regional data.

Other transfusion practices involving D variant phenotypes will be determined optimally with data and experiences that are specific for the pertinent population. Anti-D in women with the partial D phenotype has caused severe hemolytic disease of the fetus and/or newborn, but there are no universally accepted guidelines for Rh immunoprophylaxis in D variant women. Recognizing the different RHD alleles and their frequencies in different populations, we encourage additional studies (especially on the DEL phenotype) in Chinese and other Asian populations to support the development of pertinent Rh-related transfusion and obstetric practices.

REFERENCES


6. Muller TH, Wagner FF, Trockenbacher A, et al. PCR screen-
ing for common weak D types shows different distributions in three central European populations. Transfusion 2001; 40:45-52.
10. Garratty G. Do we need to be more concerned about weak D antigens? Transfusion 2005;45:1547-51.
32. Flegel WA. The above letter was also sent to Dr Flegel: Dr Flegel offered the following reply. Transfusion 2006;46:1063-4.
33. Körömoczi GF, Wagner T. The above letter was also sent to Drs Körömoczi and Wagner; Drs Körömoczi and Wagner offered the following reply. Transfusion 2006;46:1066.
35. Wagner FF, Moulds JM, Flegel WA. Genetic mechanisms of Rhesus box variation. Transfusion 2005;45:338-44.
37. Lin M. Taiwan experience suggests that RhD typing for blood transfusion is unnecessary in southeast Asian populations. Transfusion 2006;46:95-8.
39. Ohno H, Yasuda H. The above letter was also sent to Drs Ohno and Yasuda; Drs Ohno and Yasuda offered the following reply. Transfusion 2006;46:1065.