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Two distinct *RHCE* alleles in cis to *weak D type 31* alleles in individuals from different ethnicities

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In 2005, Körmöcz and colleagues reported a white blood donor with a serologic weak D phenotype (D+C+E-c+e+).¹ Her RHD gene showed a C to T nucleotide change at Position 17 (c.17C > T; rs555567027) in Exon 1, leading to the substitution of a proline for a leucine (p.Pro6Leu) in the intracellular amino-terminal domain of the *RHD* protein. Because the location of the mutation was compatible with the definition of a molecular weak D type,² this allele was assigned *weak D type 31*. The allele's description covered 50 nucleotides of the 5'-untranslated region, 148 nucleotides of Exon 1, and 47 nucleotides of Intron 1 (GenBank Accession Number [AJ557803.1](#)). Family analysis revealed a daughter typing D-C-E-c+e+ and another daughter with the weak D type 31 allele typing D+C+E-c+e+, unambiguously linking the Ce antigens in cis.¹ No further molecular variations of the involved *RHCE* allele were determined at the time.

We collected fresh weak D type 31 samples from the original donor¹ and her daughter in Germany and from a newly observed weak D type 31 donor with a D+C-c+E-e+ phenotype in India. We resolved the linkage of the involved distinct *RHD* and *RHCE* alleles in both index cases, establishing sequence information for three *RH* haplotypes. The data can be applied to develop, evaluate, and validate next-generation sequencing techniques.³ Their robustness will be increased when the allele and haplotype data conclusively include *RHD* intron polymorphisms and the linked *RHCE* allele in cis on the same chromosome.

BRIEF METHODS

DNA was extracted from ethylenediaminetetraacetate-anticoagulated whole blood samples, collected with consent from the individuals at the blood centers in Dresden, Germany, and Surat, Gujarat, India. The 6145 nucleotides of the *RHD* and 8015 of the *RHCE* gene were

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CONFLICT OF INTEREST

WAF receives royalties for *RHD* genotyping. The remaining authors have disclosed no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

sequenced as described previously.³ *RHD* zygosity testing was performed by restriction fragment length polymorphism.

RESULTS AND DISCUSSION

All three samples were hemizygous for the *RHD* gene by restriction fragment length polymorphism zygosity testing. Using our previously described RH sequencing strategy,³ we determined 14,160 nucleotides to establish three extended *RH* haplotypes.

Indian index case

RHD gene sequencing in the Indian donor revealed one single-nucleotide polymorphism (SNP), specific for the *weak D type 31* allele, in Exon 1 and five SNPs in the noncoding regions (Introns 1, 3, and 5; Table S1, available as supporting information in the online version of this paper). The *RHCE* gene sequencing identified eight homozygous SNPs in the noncoding regions. Hemizygosity at the *RHD* locus for the Indian *weak D type 31* allele combined with homozygosity at the *RHCE* locus allowed us to unambiguously identify the common *RHCE*ce* allele linked in cis (Fig. 1; GenBank Accession Number [MH260574](#)). The other *RH* haplotype represented the *RHD* deletion (*RHD*01N.01*) in cis to the common *RHCE*ce* allele (Fig. 1; GenBank Accession Number [MH260575](#)).

German family duo

The *RH* haplotype for the original donor AJ557803.1 and her daughter was determined at the molecular level. They both shared an *RHD*weak D type 31* allele with one SNP in Exon 1. Additional *RHD* intronic variations were also present in the original donor (Table S1). The *RHCE* gene sequencing identified eight heterozygous SNPs, identical to the Indian sample, and confirmed an *RHCE*Ce* allele in cis to the European *weak D type 31* allele (Fig. 1; GenBank Accession Number [MH260576](#)). The other *RH* haplotype was an *RHD*01N.01* allele in cis to the *RHCE*ce* allele with eight SNPs (Fig. 1; GenBank Accession Number [MH260575](#)), identical to the Indian sample.

Serology

The peculiar serologic phenotype of weak D type 31 has been published in detail.¹ We confirmed two red cell (RBC) populations characterized by mixed-field agglutination in tube and gel in the three cases of this study; besides the German family duo and two granddaughters, seven additional cases from Switzerland were tested in Vienna, all with D +C+E-c+e and mixed-field agglutination (data not shown). The histogram documented a negative and an adjacent weakly positive RBC population in all cases, using flow cytometry⁴ with 11 monoclonal anti-Ds as described previously.¹

Phylogeny

The one extra insertion–deletion variation (802–46_802–43delTCTC) present in the European as compared to the Indian *weak D type 31* alleles was located in *RHD* Intron 5 and has been shown to be population specific.⁵ Based on this extra SNP and the different *RH* haplotype, the Indian and European weak D type 31 alleles may have arisen in two independent events. The complete list of SNPs detected in the *RHD* and *RHCE* genes in the

Indian and German donors are tabulated as typically reported in our clinical diagnostic routine (Table S1).

We have identified three haplotypes in the *RH* system that differ from the human reference genome assembly (GRCh38), which is currently the gold standard reference.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATION:

SNP single-nucleotide polymorphism

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Fig. 1.

RH haplotype of *weak D type 31*. Both *RH* genes with 10 exons each are represented by numbered boxes for the *RHD* (yellow) and *RHCE* genes (green). The c.17C>T SNP (rs555567027; red line) in *RHD* Exon 1 represents the nonsynonymous change causing the weak D type 31 phenotype; there is no SNP in any *RHCE* exon relative to the consensus sequence. Multiple SNPs are present in the noncoding regions (black lines). Only one insertion–deletion variation (802–46_802–43delTCTC) in *RHD* but eight SNPs in *RHCE* differ between the Indian and German donors (blue arrows). The other five *RHD* variations in the promoter (–368A>G) and introns (149–29G>C, 486+117T>C, 486+124G>A and 801+219G>T) are SNPs of high prevalence commonly observed in many different *RHD* alleles, not diagnostic for the *RHD***weak D type 31* allele.