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Serologic and molecular characterization of weak D type 29

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Background

While investigating D zygosity, Perco et al.¹ found in 2003 a variant of the downstream *Rhesus box* with a novel *RHD* allele in a blood donor from a Caucasoid population. This D +C-c+E-e+ sample showed a serologic weak D phenotype. Because the serology and *RHD* structure were compatible with the definition of a molecular weak D type,² the allele was assigned *weak D type 29*. The 2918 nucleotides of the *Rhesus box* (GenBank AF469473) represented the sequence of a *downstream* connected to an upstream *Rhesus box*. A second example of weak D type 29 was observed in Tunisia among 448 samples that were D negative by the indirect antiglobulin test.³ A nucleotide sequence FR745438.1 closely resembling *weak D type 29* with 1 additional missense mutation and lacking 1 silent mutation was reported in 2010. None of these reports documented a detailed serologic description or the *RH* haplotype involved.

The nucleotide variations of *weak D type 29* were dispersed throughout the *RHD* gene and could neither be explained easily by a few mutation events nor fitted into one of the Eurasian D and African weak D type 4 clusters. We collected fresh samples from the Tunisian donor³ and the family of the original donor FR745438 to resolve both *RHD* and *RHCE* alleles and describe the serology. A new *RH* haplotype was documented.

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Conflict of interest disclosure: WAF and FFW are inventors of patents for *RHD* genotyping owned by German Red Cross Blood Service Baden-Württemberg – Hessen. The remaining authors declared having no competing financial interest relevant to this article.

Statement of Disclaimer: The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

Authorship contribution: AD and SJY collected the samples and coordinated the blood donor study; RC identified and collected samples of the trio; AD performed the serologic testing; and MO, KS and WAF analysed the serologic data. MO, AD and KS performed the molecular testing, and MO, KS and WAF analyzed the molecular data. FFW and WAF contributed tools, methods and essential reagents. MO wrote drafts of the manuscript and WAF the final version.

Study subjects and Methods

The DNA was extracted from EDTA-anticoagulated whole blood samples, collected with consent from 4 individuals at the blood centers in Sousse and Mainz. All methods have been described previously (Supplemental Methods).

Results and Discussion

Using our *RH* sequencing strategy (Fig. S1), we determined the *RH* haplotype in the Tunisian donor¹ with the weak D type 29 and found 8 SNPs in the *RHD* coding sequence (CDS), as previously described (Fig. 1);^{1,3} the serologically implicated ccDee phenotype³ was confirmed at the molecular level because the *RHCE* gene *in cis* was actually the common *RHCE*ce* allele. The other *RH* haplotype (KY617093, not shown) represented an *RHCE*ce* variant allele associated with the *RHD* deletion (*RHD*01N.01*).

Family trio

The *RH* haplotype for the original donor FR745438 and his parents were determined (Fig. S2). The father and the son shared the *RHD*weak D type 29* – *RHCE*ce* haplotype that we had observed in the Tunisian donor;³ a corrected sequence has been deposited (FR745438.2). The *RHD*weak D type 29* cDNA was confirmed in the father and the Tunisian donor.

Population

The Tunisian donor³ lived in the Gouvernorat Sousse. While exploring the family of the Caucasian donor in Mainz, Germany, the father unexpectedly reported to be born in the Gouvernorat Jendouba, Tunisia. The original weak D type 29 was described in a Caucasoid blood donor in Northern Germany,¹ of whom the ethnic origin could not be found out.

D antigen density

Both donors with the serologic weak D type 29 phenotype were tested by flow cytometry and expressed D antigen densities of 174 and 80 D antigens per red blood cell, respectively (Table S1); this is lower than the common weak D type 2 and 5 phenotypes. The additional 3 amino acid substitutions thus have a substantial suppressive effect on the membrane integration of the variant RhD protein, because weak D type 4.2 harboring the other 2 substitutions can easily express more than 1,000 D antigen per red blood cell. The observed agglutination patterns (Table S2) correlated well with the antigen densities.⁴ Very weak expression of the D antigen can interfere with the interpretation of the D epitope pattern (Table S1) and should not be misunderstood as partial D.

We also compared the published *Rhesus boxes*, analyzed the phylogeny and modeled the RhD protein (Fig. S3–S5, Supplemental Material).

Conclusion

Our study resolved 2 seemingly closely related *RHD* alleles with a complex phylogenetic origin, which was a conundrum since 2010. We established unambiguous data for 5 *RH*

haplotypes spanning a DNA stretch of more than 150,000 nucleotides each on the short arm of chromosome 1. Such data can be applied to develop, evaluate and validate next generation sequencing approaches using targeted⁵ and long range techniques.⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary statement

We describe the serology of weak D type 29 and its *RH* haplotype including stretches of the introns for more than 11,329 nucleotides. The associated downstream *Rhesus box* and genetic events leading to this *RH* haplotype are discussed. Among the 147 weak D types known so far, the *weak D type 29* haplotype has one of the most complex phylogenetic derivation.

Fig. 1. RH haplotype of weak D type 29

Both *RH* genes are represented by boxes for the 10 exons each (*RHD* gene in yellow, *RHCE* green). Among the 8 single nucleotide polymorphisms (SNPs, black lines) in the *RHD* exons, 5 represented non-synonymous and 3 synonymous changes; there is no SNP in the *RHCE* exons. In the introns and 5'-UTRs, 19 SNPs are marked (blue lines). All depicted SNPs indicate differences relative to the reference sequences for this *RHD*weak D type 29* – *RHCE*ce* haplotype (GenBank accession no. KY617089, for a total of 11,325 nucleotides, because of a 4 nucleotide deletion in *RHD* intron 2). The 1 variation in the promoter (–368A>G) and 2 variations in intron 3 (IVS3+117T>C and IVS3+124G>A) are SNPs of high prevalence commonly observed in many different *RHD* alleles and cannot be diagnostic for the *RHD*weak D type 29* allele. The *RHD*weak D type 29* is defined by the 8 nucleotide substitutions 178A>C, 201G>A, 203G>A, 594A>T, 667T>G, 744C>T, 957 G>A, 1025T>C in the exons (black lines).