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Molecular characterization of 13 new *RHD* alleles

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BACKGROUND

The D (Rh1) antigen is one of the most clinically significant blood group antigens. It is involved in transfusion and fetomaternal incompatibilities. Several hundred alleles have now been described for the *RHD* gene.[1] Some are associated with an alloimmunization risk for their carrier (partial antigen), while others are not (weak antigen).

BRIEF METHODS

Weakened D antigen expression (11 samples), unexplained mixed-field agglutination for the RhD antigen (1 sample) or the discovery of anti-D in RH:1 patients (2 samples) prompted the molecular analysis of the *RHD* gene. DNA sequencing of *RHD* exons 1 to 10 was performed by the Sanger dideoxy method using a cycle sequencing kit (BigDye Terminator v3.1, ThermoFisher, Villebon-sur-Yvette, France) and a genetic analyzer (3500Dx, ThermoFisher) according to manufacturer protocol and as previously published.[2] Since the novel alleles described are very rare compared to *RHD* deletion, zygosity testing was not performed, hemizygosity being the most likely situation.

Fresh red blood cells (RBC) were not available for serologic studies. The probands' transfusion and alloimmunization history was sought.

RESULTS

Results are summarized in Table 1.

Both probands carrying the *RHD*841C* allele had anti-D antibodies but fresh RBC were not available for adsorption techniques. Since normal *RHD* was present in trans for these patients, the anti-D were probably auto-antibodies. These two probands were the only ones with a transfusion history. They had received 2 and 8 D positive packed RBC respectively.

No other proband had or was known to have Rh antibodies.

As a precaution, we suggest that several of the novel alleles described in this report should be considered to produce partial RhD proteins. Firstly, the point mutations of alleles *RHD*119G*, *RHD*149A*, *RHD*691T, 692T* and *RHD*871T* are predicted to cause amino acid substitutions in extracellular domains, thus exposed at the RBC membrane. Secondly, the allele *RHD*394C*, is closely related to previously described alleles *RHD*394A* and *RHD*395A*, both considered to produce partial RhD proteins. [3] This should be confirmed by serologic studies when fresh RBC become available.

Novel alleles *RHD*594T, 602G, 667G, 819A* and *RHD*602G, 667G, 819A, 1063A* may be considered part of the weak D type 4 cluster, since they carry the mutations associated with *weak D type 4.0* [4] and one additional point mutation each, in positions 594 and 1063 respectively.

BRIEF SUMMARY

This report describes 13 novel *RHD* alleles predicted by serological elements and confirmed by exon genotyping.

Table 1 : New RHD alleles

Allele	GenBank	Nucleotide change(s)	Exon	Effect	N (ethnicity)	Proband phenotype	*RHCE* allele(s)	Similar alleles (involving the same amino acid) with publication reference or Genbank accession number
*RHD*41T*	KU363603	c.41C>T	1	p.Pro14Leu	1 (NA)	RH:w1,2,-3,-4,5	*RHCE*Ce*	-
*RHD*119G*	KU363605	c.119A>G	1	p.Asp40Gly (extracellular)	1 (NA)	RH:w1,2,-3,-4,5	*RHCE*Ce*	*RHD*DDE* (p.Asp40Glu) Genbank AM177313
*RHD*149A*	KU363606	c.149T>A	2	p.Val50Asp (extracellular)	1 (NA)	RH :w1,-2,-3,4,5	*RHCE*ce* or *RHCE*ce.254G*	-
*RHD*394C*	KU363608	c.394G>C	3	p.Gly132Arg	1 (NA)	RH :dp1,2,-3,4,5	*RHCE*Ce* or *RHCE*ce*	*RHD*394A* (p.Gly132Arg) *RHD*395A* (p.Gly132Glu) Silvy, 2012[3]
*RHD*691T, 692T*	KU363609	c.691C>T c.692 C>T	5	p.Pro231Leu (extracellular)	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	*RHCE*Ce* or *RHCE*ce*	-
*RHD*731T*	KU363610	c.731C>T	5	pAla244Val	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	*RHCE*Ce* or *RHCE*ce*	-
*RHD*751C*	KU363611	c.751A>C	5	p.Thr251Pro	1 (NA)	RH :w1,-2,3,4,5	*RHCE*ce* or *RHCE*cE*	-
*RHD*780A*	KU363612	c.780C>A	5	p.His260Gln	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	*RHCE*ce* or *RHCE*ce.VS.03*	-
*RHD*841C*	KU363613	c.841G>C	6	p.Val281Leu	2 (Afro-Caribbean)	RH :1,-2,-3,4,5	*RHCE*ce*	*RHD*weak D type36* (V281G) Genbank AJ867387
*RHD*871T*	KU363614	c.871C>T	6	p.Pro291Ser (extracellular)	1 (Afro-Caribbean)	RH:w1,-2,-3,4,5	*RHCE*ceVS.01*	*RHD*872G* (P291R) Genbank HE999545
*RHD*1169C*	KU363615	c.1169T>C	9	p.Leu390Pro	1 (Caucasian)	RH:w1,2,-3,-4,5	*RHCE*Ce*	-
*RHD*594T, 602G, 667G, 819A*	KU363616	c.594A>T c.602 C>G c.667T>G c.819G>A	4 4 5 6	p.Lys198Asn p.Thr201Arg p.Phe223Val p.Ala273Ala	1 (Afro-Caribbean)	RH:w1, -2,-3,4,5	*RHCE*ceVS.01* or *RHCE*ceVS.06*	*Weak D type 4.0* Wagner 1999[4]
*RHD*602G, 667G, 819A, 1063A*	KU363617	c.602C>G c.667T>G c.819G>A c.1063G>A	4 5 6 7	p.Thr201Arg p.Phe223Val p.Ala273Ala p.Gly355Ser	1 (NA)	RH:w1, -2,-3,4,5	*RHCE*ce* or *RHCE*ceVS.04* (silent point mutation in position 105)	*Weak D type 4.0* Wagner 1999[4]

aa: amino acid; NT: not tested; w: weak antigen expression; dp: unexplained mixed-field agglutination; NA: not available

REFERENCES

1. Wagner FF, Flegel WA. The rhesus site. *Transfusion Medicine and Hemotherapy.* 2014 Oct;41(5):357-63.

2. Legler TJ, Maas JH, Köhler M, Wagner T, Daniels GL, Perco P, Panzer S. RHD sequencing: a new tool for decision making on transfusion therapy and provision of Rh prophylaxis. Transfus Med. 2001 Oct;11(5):383-8.

3. Silvy M, Chapel-Fernandes S, Callebaut I, Beley S, Durousseau C, Simon S, Lauroua P, Dubosc-Marchenay N, Babault C, Mouchet C, Ferrera V, Chiaroni J, Bailly P. Characterization of novel RHD alleles: relationship between phenotype, genotype, and trimeric architecture. Transfusion. 2012 Sep;52(9):2020-9.

4. Wagner FF, Gassner C, Müller TH, Schönitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. Blood. 1999 Jan 1;93(1):385-93.