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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.<sup>1</sup> 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.<sup>2</sup> Since the novel alleles described are very rare compared to *RHD* deletion, zygosity testing was not performed, hemizyosity 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.<sup>3</sup> 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*<sup>4</sup> 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
<i>RHD*41T</i>	KU363603	c.41C>T	1	p.Pro14Leu	1 (NA)	RH:w1,2,-3,-4,5	<i>RHCE*Ce</i>	-
<i>RHD*119G</i>	KU363605	c.119A>G	1	p.Asp40Gly (extracellular)	1 (NA)	RH:w1,2,-3,-4,5	<i>RHCE*Ce</i>	<i>RHD*DDE</i> (p.Asp40Glu) Genbank AM177313
<i>RHD*149A</i>	KU363606	c.149T>A	2	p.Val50Asp (extracellular)	1 (NA)	RH :w1,-2,-3,4,5	<i>RHCE*ce</i> or <i>RHCE*ce.254G</i>	-
<i>RHD*394C</i>	KU363608	c.394G>C	3	p.Gly132Arg	1 (NA)	RH :dp1,2,-3,4,5	<i>RHCE*Ce</i> or <i>RHCE*ce</i>	<i>RHD*394A</i> (p.Gly132Arg) <i>RHD*395A</i> (p.Gly132Glu) Silvy, 2012 <sup>3</sup>
<i>RHD*691T, 692T</i>	KU363609	c.691C>T c.692 C>T	5	p.Pro231Leu (extracellular)	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	<i>RHCE*Ce</i> or <i>RHCE*ce</i>	-
<i>RHD*731T</i>	KU363610	c.731C>T	5	p.Ala244Val	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	<i>RHCE*Ce</i> or <i>RHCE*ce</i>	-
<i>RHD*751C</i>	KU363611	c.751A>C	5	p.Thr251Pro	1 (NA)	RH :w1,-2,3,4,5	<i>RHCE*ce</i> or <i>RHCE*cE</i>	-
<i>RHD*780A</i>	KU363612	c.780C>A	5	p.His260Gln	1 (Afro-Caribbean)	RH :w1,2,-3,4,5	<i>RHCE*ce</i> or <i>RHCE*ce.VS.03</i>	-
<i>RHD*841C</i>	KU363613	c.841G>C	6	p.Val281Leu	2 (Afro-Caribbean)	RH :1,-2,-3,4,5	<i>RHCE*ce</i>	<i>RHD*weak D type36</i> (V281G) Genbank AJ867387
<i>RHD*871T</i>	KU363614	c.871C>T	6	p.Pro291Ser (extracellular)	1 (Afro-Caribbean)	RH:w1,-2,-3,4,5	<i>RHCE*ceVS.01</i>	<i>RHD*872G</i> (P291R) Genbank HE999545
<i>RHD*1169C</i>	KU363615	c.1169T>C	9	p.Leu390Pro	1 (Caucasian)	RH:w1,2,-3,-4,5	<i>RHCE*Ce</i>	-
<i>RHD*594T, 602G, 667G, 819A</i>	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	<i>RHCE*ceVS.01</i> or <i>RHCE*ceVS.06</i>	<i>Weak D type 4.0</i> Wagner 1999 <sup>4</sup>
<i>RHD*602G, 667G, 819A, 1063A</i>	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	<i>RHCE*ce</i> or <i>RHCE*ceVS.04</i> (silent point mutation in position 105)	<i>Weak D type 4.0</i> Wagner 1999 <sup>4</sup>

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

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