

SEROLOGIC CHARACTERIZATION OF D ANTIGEN EXPRESSION ENCODED BY TWO REPORTED RHD ALLELES: IMPLICATIONS FOR TRANSFUSION AND PREGNANCY

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INTRODUCTION

The availability of RHD genotyping has contributed to recognition of more than 490 alleles. However, a blood sample is not always available for serologic characterization of D antigen expression encoded by a new allele. We report characterization of D on the RBCs of two females whose samples were referred for RHD genotyping. Both had rare *RHD* genes and the D serologic reactivity associated with these alleles had never been investigated.

CASES

Sample 1

- 29 year old female.
- RBCs typed C-E-c+e+.
- Variable D antigen typing results.

	ECHO	NEO	Tube testing
Immucor Series 4	1+	3+	2+/4+
Immucor Series 5	1+	‘?’	1+ ^w /4+

Sample 2

- 20 year old pregnant female.
- Predicted C-E-c+e+.
- Weak D antigen typing results.

MATERIALS AND METHODS

Serology testing

- Serologic testing was performed by automated analyzer, Galileo Echo and NEO (Immucor, Norcross, GA), and by standard tube testing with licensed anti-D reagents and the ALBAclone advanced partial RhD typing kit (Quotient, Newtown, PA).

DNA testing

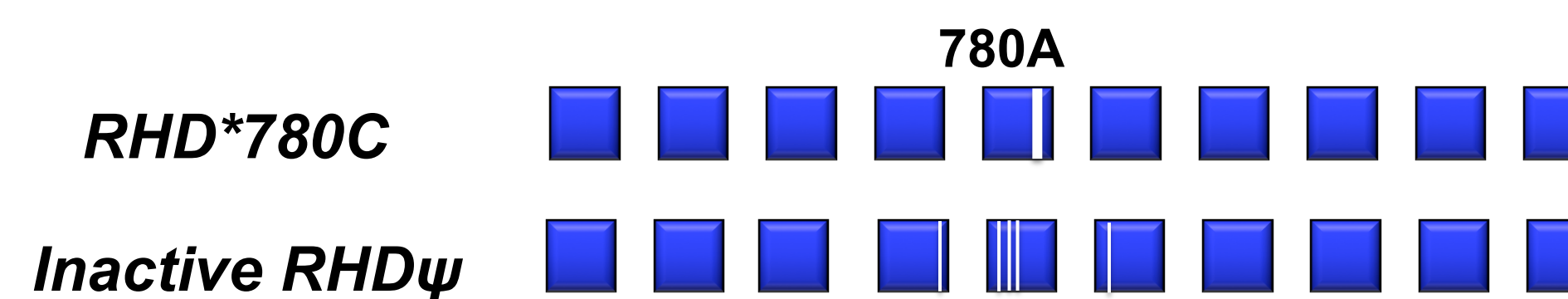
- Genomic DNA was isolated from WBCs.
- RHD* BeadChip prototype assay (BioArray/Immucor) was performed according to manufacturer's instructions.
- RHD* exons 1 to 10 were amplified and Sanger sequenced.

RESULTS

Sample 1

DNA results

- RHD* BeadChip: *RHD* / inactive *RHD* pseudogene.
- Sanger sequence:
 - Confirmed presence of *RHD* pseudogene.
 - c.780C>A change (p.His260Gln) in exon 5.
 - GenBank #KU3636120



Reactivity with multiple anti-D on initial spin and IAT (IS/IAT):

	Ortho BioClone	Bio-Rad Seraclone	Immucor Gamma-clone
Sample 1	0 / 2+	0 / 2+	+ ^w / 2+

Sample 2

DNA results

- RHD* BeadChip: *DAR* and low signal (LS) for exon 2.
- Sanger sequence:
 - Exon 2 replaced with RHCE-specific nucleotides from c.150 to c.203 (p.Val50Val to Ser68Asn).
 - Hybrid allele designated *RHD***DAR*(*CE2*:V50V-S68N).



Reactivity with multiple anti-D on initial spin and IAT (IS/IAT):

	Immucor			ALBA		
	Series4	Series 5	Gamma-clone	Alpha	Blend	Delta
Sample 2	+ ^w / 3+	+ ^w / 3+	+ ^w / 3+	2+/NA	2+/4+	2+/NA

NA = Not applicable

ALBAclone partial D IAT testing results:

Kit ID	Anti-D cell line	Wk D type 1 & 2	DII & DNU	DIII	DIV	DV	DCS	DVI	DVII	DOL	DFR	DMH	DAR	DAR-E	DHK & DAU4	DBT	R ₀ ^H	Sample 1	Sample 2
A	LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0	3+	3+
B	LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	2+ ^s	3+
C	LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	0	0
D	LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	3+	3+
E	LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	2+ ^s	1+
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	2+	3+
G	LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	2+	2+
H	LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	2+	2+
I	LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	2+	0
J	LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	2+ ^s	1+
K	LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	2+ ^s	3+
L	LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	0	+ ^w

Sample 1:

RBCs did not react with clones LHM 174/102 & 57/17.

Sample 2:

RBCs did not react with clones LHM 174/102 & 70/45.

Neither pattern matches known partial D identified by these clones.

CONCLUSIONS

- We found two previously reported rare alleles whose influence on D antigen expression has not been previously reported.
 - RHD* with a c.780C>A, p.His260Gln, was previously found in France and deposited in GenBank¹ but the serologic reactivity was never reported.
 - RHD***DAR* with part of exon 2 replaced by *RHCE*, was reported in sub-Saharan Africa² designated *RHD***DAR*(*CE2*:V50V-S68N). This allele has a frequency of 0.002 to 0.016.
- We provide serologic evidence that these alleles encode partial D phenotypes with novel epitope expression patterns. These females are predicted to be at risk for allo anti-D.

REFERENCES

- GenBank KU363612. Submitted by LeFloch et al in 2015. No publication associated with report.
- Granier et al. (2013). A comprehensive survey of both RHD and RHCE allele frequencies in sub-Saharan Africa. *Transfusion* 53(11 Suppl 2):3009-17.