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Novel *RHCE*ceS* with c.462G>T (p.Arg154Ser) Encodes Expression of a D Reactive Epitope

S Vege, J Aeschlimann, C Lomas-Francis, R. Velliquette, Z. Hu, and CM Westhoff

Immunohematology and Genomics Laboratory, New York Blood Center

BACKGROUND/CASE STUDIES

RhD typing is not always straightforward due to weak or partial D antigen expression and reagent differences.

An apparent D+ phenotype in the absence of *RHD* adds another layer of complexity. In this scenario, individuals may be at risk for clinically significant anti-D if transfused with D+ blood products and at risk of HDFN for females.

The D reactivity can be due to genetic variation in *RHCE* encoding D or D-like epitopes on RhCE protein. Examples include *RHCE*ceCF* (Crawford) and rare *RHCE*CeRN* in Blacks and *RHCE*ceSL*, **ceRT*, **ceHAR* in Caucasians.

Here, we investigated *RH* in a pregnant Hispanic woman reported to be D+ (RBCs strongly reactive with anti-D) by a commercial laboratory, but typed D– at the hospital.

MATERIALS AND METHODS

Serologic testing

- RBCs typing was performed:
  - by standard tube testing with multiple anti-D reagents
  - with ALBAclone partial D kit.

DNA/RNA testing

- Genomic DNA was isolated from WBCs.
- *RHD and RHCE* BeadChip (Immucor) was performed according to manufacturer's instructions.
- Sanger sequencing of *RHCE* exons 1 to 10 and surrounding splice sites was performed.
- RhCE-cDNA was amplification and Sanger sequenced with allele-specific primers

RESULTS

Serology results

	Immucor			Ortho	BioRad	Quotient/Albaclone			
	Gamma-Clone (GAMA401/F8D8) IS/IAT	Series 4 (MS201/MS26) IS/IAT	Series 5 (TH28/MS26) IS/IAT	Bioclone (MAD2) IS/IAT	Seraclone blend (BS232,BS22, H41 11B7) IS/IAT	Blend (LDM3/ESD1) IS/IAT	Fusion (LDM1/ESD1) IS/IAT	Alpha (LDM1) IS/37C	Delta (LDM1/ESD1M) IS/37C
Patient	3-4+/1+	0/0	+mi/0	0/0	0/0	+mi/0	0/0	+mi/0	+vw/0

- RBCs reacted 3+-4+ at immediate spin (IS) and weaker (1+) by indirect antiglobulin test (IAT) with Gammaclone anti-D.
- Her RBCs were very weakly (microscopic) reactive at IS and non-reactive at IAT with Series 5 and Albaclone blend anti-D. Microscopic reactivity was also observed with Alba alpha and delta anti-D at IS only.

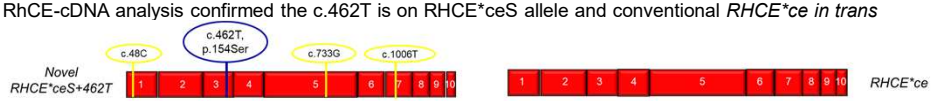
ALBA partial D kit typing results

Kit ID	Anti-D cell line	Wk D type 1 & 2	DH & DNU	DBH	DBV	DV	DCS	DV1	DVI	DOL	DFR	DMH	DAR	DAR Pr	DHK & DAU4	DBT	R_{ce}	Proband
A	LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0	0
B	LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	0
C	LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	0
D	LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	0
E	LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	0
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	0
G	LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	0
H	LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	0
I	LHM70/45	(+)/0	+	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0
J	LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	0
K	LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	0
L	LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	0

- No reactivity was observed at saline IAT with any anti-D reagents in the ALBA partial D typing kit.

DNA/RNA results

- *RHD* genotype results:
  - Positive for hybrid Rhesus box,
  - *RHD* BeadChip results: hybrid *RHD*DIIIa-CE(4-7)-D*, that types D negative.
  - No RHD gene present that encoding a D protein.
- *RHCE* BeadChip results:
  - c.48G/C, c.733C/G, and c.1006G/T consistent with *RHCE*ceS / ce*
  - *RHCE*ceS* is commonly in *cis* with hybrid *DIIIa-CE(4-7)-D*
- Sanger sequencing:
  - Confirmed c.48G/C, c.733C/G, and c.1006G/T changes.
  - Identified novel change c.462G>T (p.Arg154Ser) in exon 3 that was not found in gnomAD or dbSNP databases.
- RhCE-cDNA analysis confirmed the c.462T is on RHCE*ceS allele and conventional *RHCE*ce in trans*



CONCLUSIONS

- We report a new RHCE*ceS allele with a c.462T encoding p.154Ser.
  - The D epitope expressed by this allele is strongly reactive with Gammaclone (GAMA401) anti-D, reminiscent of the reactivity associated with *RHCE*ceCF* (Crawford).
  - That this amino acid is responsible for the reactivity with anti-D is supported by the previous report that a change at the same position, p.154Thr encoded by c.461C (*RHCE*ceRT)*, is associated with D reactivity.
- Individuals with *RHCE*ceS+462T* are suspected to be at risk for allo anti-D despite an apparent D+ phenotype with Gammaclone anti-D.
  - The patient would have been erroneously been treated as D positive and not received Rh immune globulin (RhIG) using the D typing results performed by the commercial laboratory. Although the patient's pregnancy did not go to term, she appropriately received RhIG.
- This case highlights the value of RHD genotyping in pregnancy.

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