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THREE NOVEL MISSENSE VARIANTS ASSOCIATED WITH WEAKENED D EXPRESSION

A. Huang[1], M. Alam[1], J. Fuertes[1], L. Rahorst[2], H. Lee-Stroka[1], S. Vege[1]

[1] Immunohematology and Genomics Laboratory, New York Blood Center Enterprises, Long Island City, NY

[2] Education & Training, IRL/Genomics Laboratories; New York Blood Center Enterprises, Kansas City, MO

INTRODUCTION

- The Rh blood group system is clinically significant in transfusion medicine, with the D antigen being one of the most immunogenic blood group antigens.
- Variations in the *RHD* gene can result in weak or altered D expression and complicate serologic classification.
- Resolving discrepancies is important to guide transfusion support and RhIG management.
- We report three new *RHD* alleles found in patient samples (S1-S3) sent in for *RHD* genotyping due to weak D antigen typing results

MATERIALS AND METHODS

Serology

- Serologic testing was performed by standard tube method.
- Antigen typing was done with licensed Anti-D reagents: Gamma-clone (Werfen), BioClone (Ortho), and Seraclone (Bio-Rad).

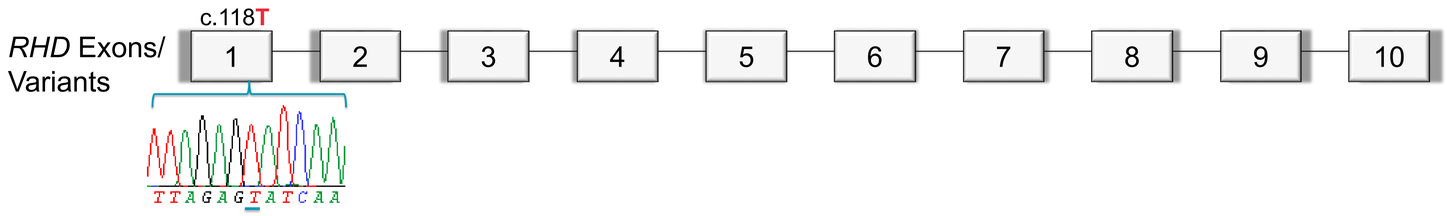
DNA

- Genomic DNA was isolated from WBCs (QIAGEN).
- *RH* exons 1-10 and flanking intron regions were sequenced by in-house developed targeted NGS (Illumina) followed by analysis using custom software
- Novel variants were confirmed by Sanger sequencing.
- Allele-specific long-range PCR (AS-LR-PCR) followed by Sanger sequencing was performed to phase the variant for S2. Specificity of products was obtained by using a reverse primer that leverages sequence associated with the *RHD*Pseudogene* allele.

RESULTS

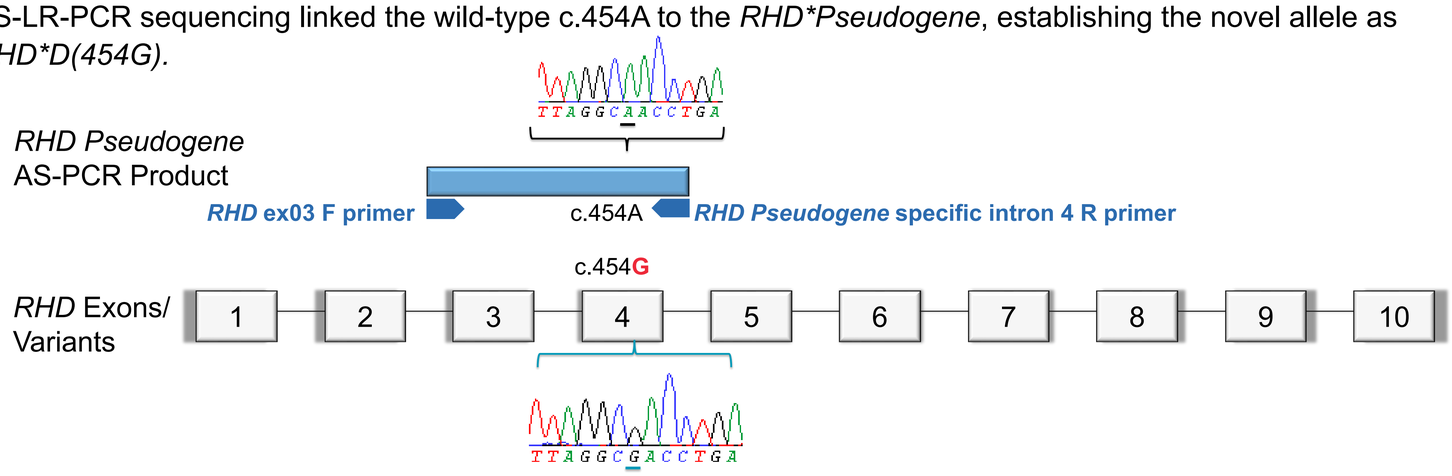
Sample 1 – White male

- RBCs were weakly reactive at IS and strongly reactive at IAT with Anti-D reagents.
- NGS identified a novel RHD with variant c.118G>T p.(Asp40Tyr) in trans to RHD deletion (*RHD*01N.01*).



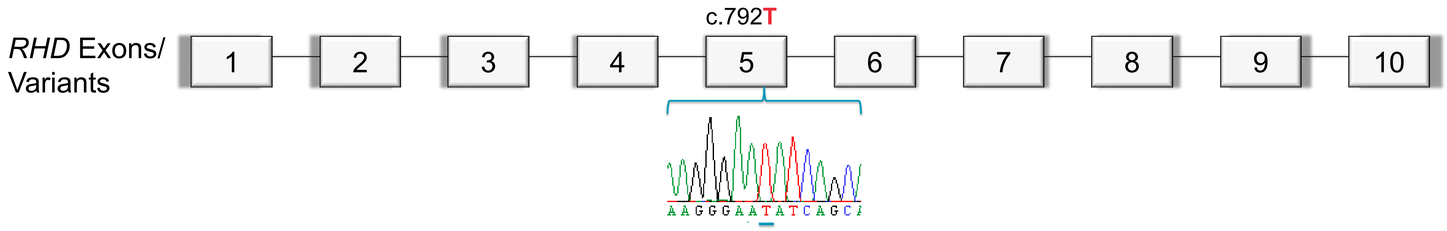
Sample 2 – Black Latino female (early gestation)

- RBCs were weakly reactive at IS and moderately reactive at IAT with Anti-D reagents.
- NGS identified variants consistent with RHD*D/RHD*Pseudogene plus variant c.454A>G p.(Asn152Asp).
- AS-LR-PCR sequencing linked the wild-type c.454A to the *RHD*Pseudogene*, establishing the novel allele as *RHD*D(454G).*



Sample 3 – White female

- RBCs were variably reactive at IS (weak to strong) with Anti-D reagents; reaction strengthened after 10-min RT incubation.
- Genotyping revealed RHD with c.792G>T p.(Lys264Asn) in trans to RHD deletion (*RHD*01N.01*).



SUMMARY

Table: Summary of serological and molecular results. Novel variants were not listed in gnomAD v.4.1.

Sample	Demographics	D Typing						Novel Variant (amino acid change)	Inferred RHD alleles (new allele in bold)
		Ortho BioClone		Bio-Rad Seraclone Blend		Werfen Gamma-clone			
		IS	IAT	IS	IAT	IS	IAT		
S1	White	2+	4+	2+	4+	2+	4+	c.118G>T p.(Asp40Tyr)	***RHD*D(118T)*** / *RHD*01N.01*
S2	Black / Latino	1+	2+	1+	3+	1+	3+	c.454A>G p.(Asn152Asp)	***RHD*D(454G)*** / *RHD*Pseudogene*
S3	White	1+/3+†	NP	4+	NP	4+	NP	c.792G>T p.(Lys264Asn)	***RHD*D(792T)*** / *RHD*01N.01*

IS = Immediate Spin; IAT = Indirect Antiglobulin Test; NP = Not Performed; † = 1+ IS, 3+ after 10min room temperature incubation

CONCLUSIONS

- We report three novel *RHD* alleles corresponding to missense variants associated with weakened/variable D antigen expression.
- All alleles were found in trans to either a *RHD* deletion (*RHD*01N.01*) or *RHD*Pseudogene*, supporting their causative role
- Notably, p.(Asp40Tyr) [c.118G>T] is predicted to reside extracellularly and p.(Asn152Asp) (c.454A>G) at the extracellular boundary of a transmembrane domain
  - Both occur at residues where alternate substitutions encode known partial D (*RHD*DDE*, p.(Asp40Glu), c.120T>A; *RHD*DNT*, p.(Asn152Thr), c.455A>C), suggesting both novel alleles also encode partial D.
- In contrast, p.(Lys264Asn) [c.792G>T] is predicted to reside intracellularly, producing possible clone-dependent variable D expression.