GENE CONVERSION WITHIN THE r'S HAPLOTYPE COMPLICATES RHD GENOTYPE INTERPRETATIONS

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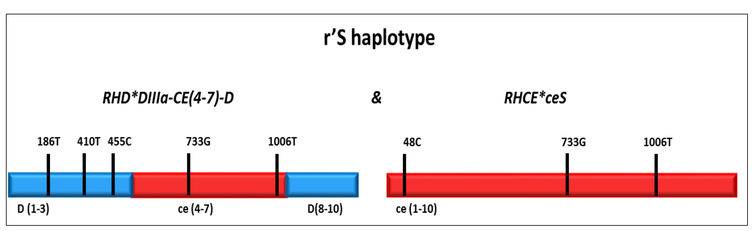
INTRODUCTION

- The homology, proximity, and inverted orientation of *RHD* and *RHCE* on the chromosome favor gene conversion events. Regions of *RHD* are transferred into *RHCE* and conversely, resulting in hybrid alleles that encode novel antigens or the absence of high prevalence antigens.
- The most common *RH* hybrid allele is *RHD*DIIIa-CE(4-7)-D*, found in African Blacks. It arose by conversion of exons 4-7 of the *RHCE*ceS* allele into *RHD*DIIIa.*
- This hybrid allele is often found in cis to *RHCE*ceS* and together they are known as the r'S haplotype, where
  - *RHD*DIIIa-CE(4-7)-D* no longer encodes D antigen but rather, somewhat confusingly, encodes partial C antigen from the *RHD* locus and
  - *RHCE*ceS* encodes partial c and e antigens.



CASES

- **Case1**
- Male patient, referred for D variant testing as RBCs typed weaker than expected.
- **Case 2**
- Multiracial female, referred for D variant testing as RBCs typed 0-1+ on Echo automated system.
- **Case 3**
- SCD African American male, referred for *RH* genotyping.

MATERIALS AND METHODS

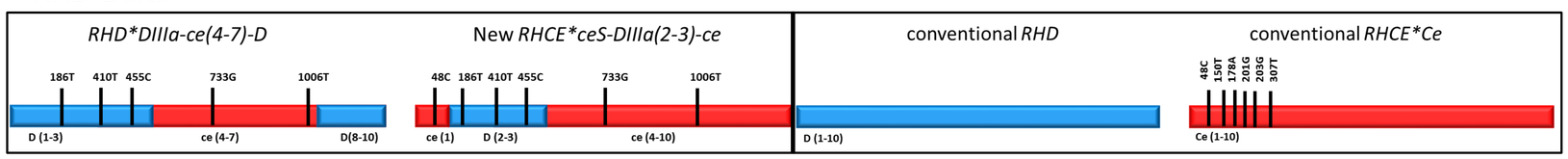
- **Serologic testing**
- Was performed by standard tube methods.
- **Molecular testing**
- Genomic DNA was isolated from WBCs from peripheral blood samples.
- All samples were investigated by HEA PreciseType, *RHD* and *RHCE* BeadChip and PCR-RFLP.
- RNA was isolated from RBCs and cDNA obtained by reverse transcription reaction.
- cDNA was amplified with *RHD* and *RHCE* specific primers and full length products (exons 1-10) sequenced.
- SNP-specific sequencing primers were used to establish linkage/phasing.

RESULTS CASE 1 & 2

- **Serologic results**
- RBCs typed C+E−c−e+ (presumed R_1R_1) and presented with weaker than expected D typing with multiple anti-D reagents.

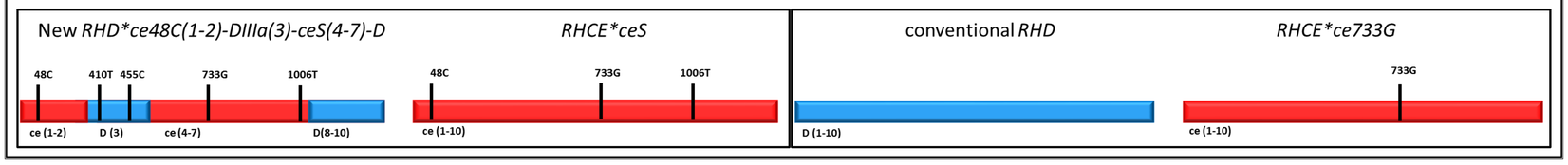
sample	Anti-D Ortho BioClone (IS/IAT)	Anti-D Immucor series 4 (IS/IAT)	Anti-D Immucor series 5 (IS/IAT)	Anti-D Immucor gammaclone (IS/IAT)	Anti-D BioRad Seraclone (IS/IAT)
1	1+/4+	1+/4+	1+/4+	$1+^s$/4+	NA
2	1+/2+	NT	NT	1+/3+	$2+^s$/NA

- ***RH* DNA results**
- *RHD* BeadChip: common African *RHD*DIIIa-CE(4-7)-D* hybrid encoding partial C with apparent conventional *RHD* in trans.
  - ***These results did not provide an explanation for weak D antigen*.**
- HEA: C+E−c−e+, concordant with phenotype, but possible variant (PV) for V/VS (c.733C/G,1006G/T heterozygous).
- *RHCE* BeadChip: *RHCE*Ce/Ce* with c.733C/G and c.1006G/T heterozygous.
  - ***Since c.733G and c.1006T together have not been observed on RHCE*Ce, further testing was indicated*.**
- ***RH* cDNA results**
- *RHD* cDNA transcripts: *RHD* and *RHD*DIIIa-ce(4-7)-D*.
- *RHCE* cDNA transcripts: *RHCE*Ce* and a new hybrid, specifically *RHCE*ceS* with exons 2 and 3 replaced with those from *RHD*DIIIa*.



RESULTS CASE 3

- **Serologic results**
- RBCs typed D+C−E−c+e+ (presumed R_0R_0/R_0r).
- **RH DNA results**
- HEA: C−E−c+e+ and V/VS+ (c.733G/G homozygous and c.1006G/T heterozygous).
- *RHCE* BeadChip: *RHCE*ce733G/ceS.*
- *RHD* BeadChip: *RHD* and *RHD*DIII type 8*.
  - ***RHD*DIIIa type 8* has never been found with either of these *RHCE* alleles, prompting further investigation*.**
- **RH cDNA results**
- *RHCE* cDNA transcripts: confirmed *RHCE*ce733G* and *RHCE*ceS.*
- *RHD* cDNA transcripts: *RHD* and an allele representing a unique conversion event at the *RHD* locus, specifically *RHCE*ce(48C)* exons 1 and 2 had replaced the corresponding exons in the hybrid *RHD*DIIIa-CE(4-7)-D* and expression of partial C antigen was lost.



CONCLUSIONS

- We report two novel complex *RH* rearrangements, both originating on a r'S haplotype:
  - two samples (1 and 2) thought to be R_1R_1 had a unique *RHCE* locus representing a gene conversion into *RHCE*ceS*, proposed designation ***RHCE*ceS-DIIIa(2-3)-ce***.
  - one sample (3) genotyped as *DIII type 8* had a novel *RHD* locus representing a gene conversion into the common hybrid, proposed designation ***RHD*ce48C(1-2)-DIIIa(3)-ceS(4-7)-D***.
- These represent novel events on the r'S haplotype that can confound *RH* genotyping interpretations, highlighting the importance to further investigate samples with unconventional results when interpreting *RH* genotype.
- Interestingly, samples 1 and 2 have weaker than expected D antigen typing, despite the presence of a conventional *RHD* with *RHCE*Ce* [R_1 haplotype (DCe)]. We hypothesize that the hybrid gene product may interfere with trafficking of RhD.