A novel RHCE*02 allele, containing the single-nucleotide change c.460A>G, encodes weakened expression of C and e antigens

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A novel *RHCE*02* allele, containing the single nucleotide change c.460A>G, encodes weakened expression of C and e antigens

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Abstract:

We report a novel *RHCE*02* allele in a Swedish blood donor that is characterized by the change; c.460A>G (Arg154Gly). The blood donor's RBCs showed variable reactivity with different monoclonal anti-C and anti-e and antigen strength was markedly weakened. We believe these changes represent both a quantitative and qualitative alteration of the antigens encoded by this allele.

Background: Rh blood group antigens are carried on two similar multipass membrane proteins encoded by *RHD* and *RHCE*. *RHD* encodes D and several partial D-associated low prevalence antigens whereas *RHCE* encodes C, E, c, e as well as many other antigens of high or low prevalence. In addition, mutations/polymorphisms in the genes may affect the level of protein expression.[1]

Case Study and Aims: Routine Rh phenotyping on samples from a group A RhD positive Swedish blood donor revealed the following results: C+^{w}c+E+e+^{w}. A follow-up sample was requested at the time of her next donation. At this time, her red blood cells (RBCs) typed C−c+E+e+^{w}. The aim of this study was to investigate the molecular basis of the *RHCE* gene and to further characterise the C and e antigens serologically.

Materials and Methods: The donor's RBCs were tested with a panel of 6 monoclonal anti-C and 6 anti-e by standard serological techniques. Routine in-house allele-specific PCR of *RHD/RHCE* was performed. *RHCE* exons 1-10 were sequenced following by exon-specific amplification (ABI BigDye v3.1, Life technologies, Carlsbad, CA, USA); and analysed using CodonCode Aligner (CodonCode Co., Centerville, MA, USA).

Results: Tests with anti-C revealed weak reactivity only with one reagent, which contained the MS273 clone; while tests with anti-e revealed weak reactivity with one of two anti-e reagents that contained a blend of MS16/MS21/MS63 clones and a 4+ reaction with anti-e containing clone BS260. The other reagents were nonreactive (Table 1). Allele-specific PCR revealed an apparent *RHCE*02/RHCE*03* genotype, consistent with the C+c+E+e+ phenotype. Sequence analysis revealed heterozygosity for a novel mutation, c.460 A>G, in exon 3, which encodes a change of Arg154Gly. Further sequence analysis was performed on a PCR product amplified with an intron 2 *RHCE*C*-specific primer to confirm that the mutation was carried on the

*RHCE*02* allele. The sequence was deposited in GenBank (KU744002) and assigned the number *RHCE*02.26* by the ISBT working party for Red Cell Immunogenetics and Blood Group Terminology. The SNP was found in dbSNP (rs755299894) where it had been found in 2/60706 individuals in the ExAC Browser (http://exac.broadinstitute.org/).

Discussion and Conclusions: We describe a novel *RHCE*02* allele that gives rise to an altered expression of both the C and e antigens. Residue 154 is associated with the start of the 5th transmembrane helix and it is likely that a change from arginine to glycine could affect how the protein is inserted into the RBC membrane, however this remains unproven in the absence of expression studies The amino acid polymorphisms responsible for C and e antigens are encoded by exon 2 and exon 5, respectively and are thus not directly affected but transmembrane alterations in the similar RhD protein have been shown to cause both quantitative and qualitative changes to RhD.[2] The failure of the RBCs to react with 4 of 6 monoclonal anti-e, together with the strong positivity with anti-e clone BS260 and weaker reactivity with one of the two monoclonal reagent blends, suggests that the e antigen expressed by this allele is a partial antigen. This raises the question of whether this donor is at risk of producing anti-e if challenged. This question was answered in part by analysis of samples from a patient, also of Swedish descent, whose RBCs originally typed C−c+E+e+^{w} and whose plasma contained an alloanti-e. DNA analysis of *RHCE* revealed heterozygosity for *RHCE*C/c* and *RHCE*E/e* as well as c.460A/G. While further samples could not be obtained for additional serological testing, *RHCE*C*-specific sequence analysis confirmed that c.460G was carried on this allele. We conclude that an *RHCE*02* allele containing the change c.460A>G gives rise to C and e antigens that are both qualitatively and quantitatively altered, and that individuals with this allele risk being not only mistyped for these antigens but at risk of producing alloanti-e.

References:

1. Daniels G. Human Blood Groups. 3 ed. Oxford: Wiley-Blackwell, 2013.

2. Westhoff CM. Rh complexities: serology and DNA genotyping. Transfusion 2007;**47**: 17S-22S.

Table 1: Reactivity of the blood donor's RBCs with a panel of monoclonal anti-C and anti-e.

Reagent	**Anti-C**						**Anti-e**					
Source	Immucor	Diamed	Diagast	Ortho	Biotest	Immucor	Diagast	Diagast	Biotest	Immucor	Immucor	Biorad
Clone/ID	MS24	id-nr 10600	MS24	lot CB259A	392/ P3x25513GB	MS273	MS16/MS63	BS267/ 16639-11	BS260/267	MS16/MS21 /MS63	MS62/MS69	MS16/MS21 /MS63
Pos. cont.	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Neg. cont.	0	0	0	0	0	0	0	0	0	0	0	0
Blood Donor	0	0	0	0	0	3+	0	0	4+	0	0	2+