RHCE variant allele: *RHCE*ce254G,733G*

J.A. Keller, T. Horn, C. Chiappa, C. Melland, C. Vietz, L. Castilho, and M.A. Keller

A novel *RHCE* allele was identified in a 53-year-old African-American female blood donor with an Rh phenotype of D+ C– E– c+ e+ and a negative antibody screen. The donor's cells typed e+ with all antisera tested. By gel-based genotyping and cDNA analysis, the two *RHCE* alleles in this donor were characterized. One allele was found to be the known allele *RHCE*01.20.01 (RHCE*ce733G)* and the second was novel: *RHCE*01.06.02 (RHCE*ce254G,733G).* ***Immunohematology*** **2014;30:121–122.**

Case Report

A novel *RHCE* allele, *RHCE*ce254G,733G,* was identified in a 53-year-old African-American female blood donor with an Rh phenotype of D+ C– E– c+ e+ and a negative antibody screen. The donor's cells typed e+ with all antisera tested. The anti-e reagents tested were Seraclone (formulated from clones MS16/MS21/MS63) (Bio-Rad Laboratories, Hercules, CA), BioClone (formulated from MS16 clone) (Ortho Clinical Diagnostics, Rochester, NY), Immucor Series 1 (formulated from MS16 clone), and Immucor Gamma-clone (formulated from clones MS16/21/63) (Norcross, GA). The hr^B status was not assessed serologically.

Materials and Methods

Genomic DNA from the donor was isolated from white blood cells using the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) and was used for genotyping of the *RHD* and *RHCE* genes using laboratory-developed gel-based genotyping assays including a multiplex *RH* polymerase chain reaction (PCR) to detect *RHD* intron 7, exon 4, *RHD* pseudogene and *RHCE* expressing the C or c antigens, restriction fragment-length polymorphism (RFLP) PCR, and sequence-specific primer (SSP) PCR. Red cell RNA isolation was performed by a laboratory-developed phenol-chloroform extraction and isopropanol precipitation method, cDNA synthesis with a Superscript III Synthesis kit (Life Technologies, Carlsbad, CA), reverse transcription–PCR amplification using *Taq* polymerase (Qiagen), and PCR product cloning with a TOPO TA cloning kit (Life Technologies). cDNA sequence analysis (cDNA seq) was performed (BigDye Terminator Kit, Life Technologies) using vector-based primers and compared with the consensus sequence using Sequencher 5.0 (GeneCodes Corporation, Ann Arbor, MI).

Results

A variety of gel-based genotyping assays were used to screen the donor sample for known single nucleotide polymorphisms (SNPs). The results are summarized in Table 1. cDNA analysis was performed to further characterize the two *RHCE* alleles to rule out the presence of additional variants. One allele was found to be known: *RHCE*01.20.01 (RHCE*ce733G).* The second allele has not been reported previously; it carries two SNPs: *RHCE* c.254G and c.733G. The allele was named *RHCE*ce254G,733G* (Table 2) based on the ISBT allele terminology.[1]

Table 1. *RH* genotyping results

Gene or Gene Region	Method	Analyte	Result	Reference
RHD gene	Multiplex PCR	*RHD* Intron 4	present	5
		RHD Exon 7	present	
		Pseudogene	absent	
		C	absent	
		c	present	
	PCR	Hybrid Rhesus box	absent	6
RHD Exon 3	SSP	455A>C (N152T)	A	7
RHD Exon 5	RFLP	667T>G (F223V)	T	7
	RFLP	697G>C or A (E233Q or E233K)	G	7
RHD Exon 8	RFLP	1136C>T (T379M)	C	8
RHCE Exon 1	RFLP	48G>C (W16C)	G	9
RHCE Exon 2	RFLP	254C>G (A85G)	C/G	10
RHCE Exon 5	RFLP	676G>C (A226P)	G	11
	RFLP	733C>G (L245V)	G	12
RHCE Exon 7	SSP	1006G>T (G336C)	G	12
	RFLP	1025C>T (T342I)	C	13
RHCE	cDNA seq	cDNA	733G (plasmid type 1)	14
			254G, 733G (plasmid type 2)	

Table 2. Allele characteristics

Phenotype	Allele name	Nucleotide	Exon(s)	Amino acid(s)	Allele detail	Reference
Possible hr^{B}–	*RHCE*01.06.02*	254C>G (rs57992529)	2	85 Ala>Gly	*RHCE*ce254G,733G*	2
		733C>G (rs1053361)	5	245 Leu>Val		

Additional Information

This case was the topic of a poster at the International Society of Blood Transfusion International Congress.[2] As per the National Center for Biotechnology Information,[3] the minor allele frequency of *RHCE* c.254G and c.733G are 0.0078 and 0.0680, respectively. The frequency of the newly identified allele *RHCE*ce254G,733G* is unknown, but, based on the frequency of the two SNPs, is expected to be rare. RFLP-PCR with *Hae*II can be used to interrogate *RHCE* c.254 for the presence of the variant.[4] Although the phenotype encoded by this allele has not been fully investigated, it is noteworthy that the *RHCE*01.06* allele with only c.254G encodes a partial e antigen and an RH:–59 (CEAG–) and RH:–31 (hrB–) phenotype.

Acknowledgments

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Jessica A. Keller, MS, Lead Molecular Technologist, and Trina Horn, MT(ASCP), MS, Supervisor, American Red Cross, National Molecular Laboratory; Colleen Chiappa, Bonfils Blood Center; Camilla Melland, Bonfils Blood Center, Denver, CO; Christine Vietz, Cambridge, MA; Lilian Castilho, Hemocentro-Unicamp, R. Carlos Chagas, Campinas-SP, Brazil; and Margaret A Keller, PhD (corresponding author), Director, American Red Cross, National Molecular Laboratory, 700 Spring Garden Street, Philadelphia, PA 19123.