An update on the RHAG blood group system

Louise A. Tilley

International Blood Group Reference Laboratory, NHS Blood and Transplant, Filton, Bristol, UK
louise.tilley@nhsbt.nhs.uk

This update on the RHAG blood group system (ISBT 030) (Chou ST, Westhoff CM. The Rh and RhAG blood group systems. Immunohematology 2010;26:178–86) reports the addition of three new low-prevalence antigens carried on the Rh-associated glycoprotein (RhAG). Kg (previously 700045; now RHAG5) has been demonstrated to be antithetical to the previously described high-prevalence DSLK (RHAG3). Two further low-prevalence antigens (RHAG6 and RHAG7) are described, both resulting from rare missense *RHAG* mutations encoding amino acid changes predicted to be externally located. All three new low-prevalence antigens have been implicated in hemolytic disease of the fetus and newborn. The RHAG system now comprises six antigens, two of high prevalence and four of low prevalence, including one antithetical pair. RHAG4 has been made obsolete. ***Immunohematology* 2025;41:1–3. DOI: 10.2478/immunohematology-2025-002.**

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New Antigens in the RHAG System

Since publication of the original review in 2010,[1] the RHAG system has expanded, with the addition of three new low-prevalence antigens, to comprise six antigens in total (two of high prevalence and four of low prevalence) (Table 1). RHAG4 was made obsolete in 2018,[2] after investigations showed that the *RHAG* variant c.808G>A (p.Val270Ile) was a common polymorphism[3] and did not encode a low-prevalence antigen.

In 2020, a proteomics approach was used to demonstrate that the low-prevalence, 700-series antigen Kg (previously 700045), first reported in a case of hemolytic disease of the fetus and newborn (HDFN) more than 30 years previously,[4] was carried on RhAG.[5] This case demonstrated that a Kg+ HDFN-affected infant in Japan, together with his older twin siblings and father, were all heterozygous for *RHAG* c.490A>C (rs144305805; p.Lys164Gln), the same single nucleotide variant as found in the homozygous state in the RHAG:-3 (DSLK–) propositus.[6] Expression studies in Chinese Hamster Ovary cells confirmed this variant to be responsible for Kg expression,[5] and Kg (now RHAG5) and DSLK have since been confirmed to be antithetical.[7]

The low-prevalence antigen RHAG6 (SHER) was identified through the course of an investigation into a case of mild HDFN. Paternal sequencing revealed the rare *RHAG* variant c.1063A>C (rs1187324502; p.Asn355His)[8] which was

Table 1. Genetic basis of RHAG blood group system antigens

Antigen number	Antigen name	Antigen prevalence	Nucleotide change	Exon	Amino acid change	rs number	Global frequency	Highest population frequency	Other information
RHAG1	Duclos	High	c.316C (G)	2	p.Gln106 (Glu)	1180686517	0.00001	0.000015 (EUR)	
RHAG2	Ol[a]	Low	c.680C>T	5	p.Ser227Leu	902283342	0.000001	0.0000017 (EUR)	Homozygosity results in Rh_{mod} phenotype
RHAG3*	DSLK	High	c.490A (C)	3	p.Lys164 (Gln)	144305805	0.00001	0.0005 (EAS)	HDFN, reported in two families[4,5]
RHAG5*	Kg	Low	c.490A>C	3	p.Lys164Gln				
RHAG6	SHER	Low	c.1063A>C	7	p.Asn355His	1187324502	0.000003	0.000004 (EUR)	Mild HDFN, single case[8]
RHAG7†	THIN	Low	c.140T>C	1	p.Phe47Ser	2127360274	0.000005	0.00066 (SAS)	HDFN, single case[9,10]

Nucleotide and amino acid residues in parentheses (for high-prevalence antigens) are associated with the antigen-negative phenotype. Frequencies refer to the minor allele in all cases.

*RHAG3 and RHAG5 are antithetical.

†RHAG7 has provisional status.

EUR = European (non-Finnish); EAS = East Asian; HDFN = hemolytic disease of the fetus and newborn; SAS = South Asian.

inherited by the affected infant and the previous unaffected child. This variant is predicted to encode an amino acid substitution located within the sixth extracellular loop of RhAG.

The final low-prevalence antigen RHAG7 (THIN) was discovered during serologic and molecular investigation of a case of HDFN in a Thai infant. Using targeted next-generation sequencing, the infant, father, and older sibling were all found to carry a c.140T>C (rs2127360274; p.Phe47Ser) substitution in *RHAG*.[9,10] RHAG7 currently has provisional status while awaiting expression studies for confirmation.

Figure 1 shows a schematic of the RhAG protein in the membrane, with the positions of the amino acid substitutions underlying the six RHAG system antigens highlighted in the external protein loops.

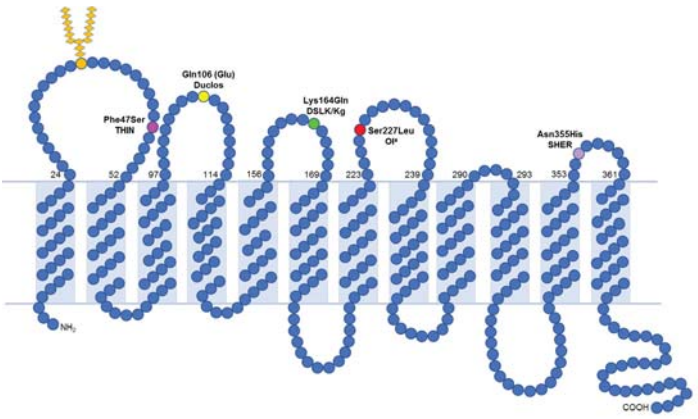

Fig. 1 Schematic of RhAG protein in the membrane. Positions of known antigenic sites in external loops are depicted by different colored circles. The position of the *N*-glycan (Asn37) in the first external loop is shown in orange. Predictions of topology of RhAG protein in the membrane vary because of a lack of a defined crystal structure; this model is based on predictions from the human transmembrane proteome. From Dobson et al.[11]

Rh_{mod} and Rh_{null}

An increase in the use of molecular methods and large-scale sequencing technologies has led to the recent description of multiple novel *RHAG* alleles. Fifteen alleles (*RHAG*01M.01–RHAG*01M.15*),[12] mostly characterized by missense mutations, have now been described to result in the Rh_{mod} phenotype. Homozygosity, or compound heterozygosity, for an $RHAG_{mod}$ allele results in markedly weakened expression of RhAG, RhD, and RhCcEe. Similarly, the number of *RHAG* alleles resulting in the Rh_{null} phenotype also continues to increase, with 27 alleles listed by the International Society of Blood Transfusion to date.[12] These alleles carry a mixture of missense and nonsense mutations, as well as splice-site mutations and indels. Homozygosity, or compound heterozygosity, for an $RHAG_{null}$ allele results in a complete lack of RhAG, RhD, and RhCcEe expression on the red blood cell (RBC).

RhAG Structure and Function

RhAG has a high level of homology with RhD and RhCE, and the proteins share the same membrane-spanning topology, comprising 12 transmembrane domains and 6 extracellular loops, although RhAG protein is glycosylated on the first external loop (Fig. 1). RhAG is closely associated with the Rh proteins in the RBC membrane, forming heterotrimers consisting of two monomers of RhAG and one of RhD or, more usually, RhCE.[13,14] The Rh heterotrimer has been shown to act as the primary membrane attachment site for ankyrin-1, mediated via the N- and C-termini of RhCE and stabilized by RhAG.[14] Ankyrin-1 is at the heart of an erythrocyte protein macrocomplex, also comprising band 3 anion exchanger, protein 4.2, and glycophorins A and B. This complex acts to tether spectrin to the membrane, playing a vital role in maintaining the size, structure, and shape of the RBC.[14] In addition to its structural role, RhAG acts as a gas channel, mediating transport of ammonium, and possibly CO_2, across the erythrocyte membrane.[15,16] Mutations in *RHAG* are associated with overhydrated hereditary stomatocytosis,[17] and deficiency of RhAG in the Rh_{null} phenotype is also associated with abnormal cell morphology and increased cell fragility, highlighting the important role RhAG plays in the erythrocyte.

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