Rh-Associated Glycoprotein Blood Group System

Number of antigens 4

Low prevalence	Ol[a], RHAG4
High prevalence	Duclos, DSLK (Duclos-like)

Terminology

ISBT Symbol (number)	RHAG (030)
CD number	CD241
History	RhAG was elevated to a blood group system in 2008 when the molecular basis of Duclos, Ol[a], and DSLK were determined to be due to nucleotide changes in *RHAG*.

Expression

Cord RBCs	Expressed
Tissues	RhAG homologs

Gene

Chromosome	6p21.3
Name	*RHAG*
Organization	10 exons distributed over 32 kbp of DNA
Product	Rh-associated glycoprotein (RhAG)

The Blood Group Antigen (3/e). DOI: http://dx.doi.org/10.1016/B978-0-12-415849-8.00032-6

Database accession numbers

GenBank	X64594 (mRNA); NG_011704 (gene); NM_000324 (mRNA)
Entrez Gene ID	6005

Molecular basis of Rh-associated glycoprotein antigens[1]

Reference allele, *RHAG*01* (Accession number X64594) encodes Duclos (RHAG1), RHAG3. Differences from this allele are given.

Allele encodes	Allele name	Exon	Nucleotide	Amino acid	Ethnicity (prevalence)
Duclos− or RHAG:−1	*RHAG*−01*	2	316C>G	Gln106Glu	(Rare)
Ol(a+) or RHAG:2	*RHAG*01.02*	5	680C>T^	Ser227Leu	Norwegian, Japanese (Several)
DSLK− or RHAG:−3	*RHAG*01.−03*	3	490A>C	Lys164Gln	(Rare)
RHAG:4	*RHAG*01.04*	6	808G>A	Val270Ile	(Rare)

^= Homozygosity for *RHAG*01.02* encodes an Rh_{mod} phenotype.

Molecular bases of silencing of *RHAG*

Homozygosity and compound heterozygosity leads to regulator Rh_{null} phenotype.

Assignment of null (*N*) alleles has been made (by the ISBT working party) according to the lack of phenotypic expression of RhD and RhCE antigens or lack of reactivity with monoclonal anti-RhAG (e.g., 2D10, L18.18). Differences from *RHAG*01* reference allele (Accession number X64594) are given.

Allele name	Exon (intron)	Nucleotide	Restriction enzyme	Amino acid	Ethnicity (prevalence)
*RHAG*01N.01*	2	154–157CCTC>GA	*Mnl*I–	Tyr51fs, Ile107Stop	South African (Rare)
*RHAG*01N.02*	8	1086delA	*Pvu*II–	Ala362fs, Val374Stop	(Rare)
*RHAG*01N.03*	Intron 1	IVS1+1g>a		Alternative splicing	White American (Rare)
*RHAG*01N.04*	Intron 6	IVS6+1g>a		Alternative splicing[2]	(Rare)
*RHAG*01N.05*	Intron 6	IVS6−1g>a		Alternative splicing	Spanish (Rare)
*RHAG*01N.06*	Intron 6	IVS6−1g>t		Alternative splicing	Japanese (Rare)
*RHAG*01N.07*	Intron 7	IVS7+1g>a	*Pml*I–	Alternative splicing	Japanese (Rare)
*RHAG*01N.08*	6	808G>A^ 838G>A		Val270Ile Gly280Arg	Japanese (Rare)
*RHAG*01N.09*	6	836G>A	*Mnl*I–	Gly279Glu	Australian (English/ French, Irish/Scottish) (Rare)
*RHAG*01N.10*	8	1094T>G		Leu365Arg[2]	(Rare)
*RHAG*01N.11*	9	1139G>T		Gly380Val + alternative splicing	Japanese (Rare)
*RHAG*01N.12*	5	762C>A		Ser224Arg[3]	Chinese (Rare)

See Rh blood group system for molecular bases of amorph Rh_{null} phenotype.
^= This single change encodes RHAG:4.

Molecular bases of weak Rh-Associated Glycoprotein antigens

Homozygosity, compound heterozygosity, or heterozygosity for *RHAG*01M.08* or *RHAG*01M.09* leads to Rh_{mod} phenotype.
Assignment of mod (*M*) alleles has been made (by the ISBT working party) according to the weak phenotypic expression of RhD and RhCE antigens or depressed RhAG.
Differences from *RHAG*01* reference allele (Accession number X64594) are given.

Allele name	Exon	Nucleotide	Amino acid	Ethnicity (prevalence)
*RHAG*01M.01*	9	1183delA	Asn395fs + 52 additional amino acids	Japanese (Rare)
*RHAG*01M.02*	1	3G>T	Met1Ile	Jewish Russian (Rare)
*RHAG*01M.03*	2	236G>A	Ser79Asn	Caucasian (Rare)
*RHAG*01M.04*	2	269G>T	Gly90Val[4]	(Rare)
*RHAG*01M.05*	3	398T>C	Leu133Pro[5#]	(Rare)
*RHAG*01M.06*	4	560G>A	Gly187Asp[4]	(Rare)
*RHAG*01M.07*	9	1195G>T	Asp399Tyr	French (Rare)
*RHAG*01M.08*	2	182T>G	Ile61Arg	(Rare)
*RHAG*01M.09*	2	194T>C	Phe65Ser	(Few)
*RHAG*01.02^*	5	680C>T^	Ser227Leu	Japanese

^= Homozygosity causes a Rh_{mod} phenotype. This allele encodes the Ol^a antigen.
#= In the abstract 133 is, incorrectly, given as Arg.

Amino acid sequence

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MRFTFPLMAI  VLEIAMIVLF  GLFVEYETDQ  TVLEQLNITK  PTDMGIFFEL   50
YPLFQDVHVM  IFVGFGFLMT  FLKKYGFSSV  GINLLVAALG  LQWGTIVQGI  100
LQSQGQKFNI  GIKNMINADF  SAATVLISFG  AVLGKTSPTQ  MLIMTILEIV  150
FFAHNEYLVS  EIFKASDIGA  SMTIHAFGAY  FGLAVAGILY  RSGLRKGHEN  200
EESAYYSDLF  AMIGTLFLWM  FWPSFNSAIA  EPGDKQCRAI  VDTYFSLAAC  250
VLTAFAFSSL  VEHRGKLNMV  HIQNATLAGG  VAVGTCADMA  IHPFGSMIIG  300
SIAGMVSVLG  YKFLTPLFTT  KLRIHDTCGV  HNLHGLPGVV  GGLAGIVAVA  350
MGASNTSMAM  QAAALGSSIG  TAVVGGLMTG  LILKLPLWGQ  PSDQNCYDDS  400
VYWKVPKTR                                               409
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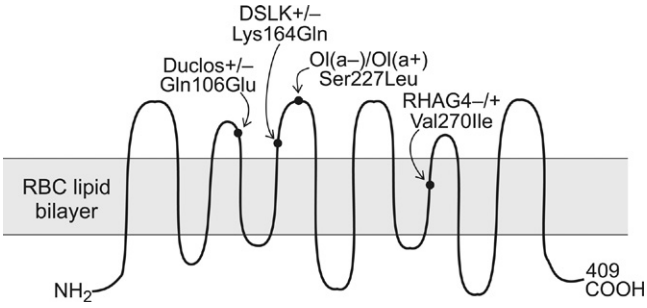
Carrier protein

A multipass membrane glycoprotein.



M_r (SDS-PAGE)	45,000 to 100,000 with a predominant band of 50,000
CHO: N-glycan	1 (plus 1 potential site)
Cysteine residues	5
Copies per RBC	100,000–200,000

Function

RhAG forms a core complex with RhD and/or RhCE. This complex is also associated with GPB, LW, and CD47, which in turn is associated with the band 3/GPA complex that links to the RBC membrane skeleton via ankyrin and protein 4.2. This complex maintains erythrocyte membrane integrity, as demonstrated by the abnormal morphology of stomatocytic Rh_{null} RBCs. Involved in NH_4^+/NH_3 or CO_2/O_2, and cation transport across the membrane[6].

Disease association

Compensated hemolytic anemia occurs in some individuals with regulator type Rh_{null} or Rh_{mod} RBCs. Over-hydrated stomatocytosis (OHSt) is associated with dominant mutations in RhAG: Phe65Ser (six families) and Ile61Arg (one family), and cation leak[7].

Comments

RhAG is essential for expression of Rh antigens[8].

References

[1] Tilley, L., et al., 2010. A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein. Vox Sang 98, 151–159.

[2] Tsuneyama, H., et al., 2005. Identification of two new mutations in the RhAG gene of Japanese with Rh_{null} phenotype [abstract]. Transfusion 45 (Suppl.), 130A.
[3] Tian, L., et al., 2011. A family study of the Chinese Rh(null) individual of the regulator type: a novel single missense mutation identified in RHAG gene. Transfusion 51, 2686–2689.
[4] Scharberg, A., et al., 2006. RHMOD phenotype caused by double heterozygosity for two new alleles of the RHAG gene [abstract]. Vox Sang 91 (Suppl. 3), 129.
[5] Tsuneyama, H., et al., 2008. Identification of two new mutations in the RhAG gene of Japanese with Rh_{mod} phenotype [abstract]. Transfusion 48 (Suppl.), 185A–186A.
[6] Burton, N.M., Anstee, D.J., 2008. Structure, function and significance of Rh proteins in red cells. Curr Opin Hematol 15, 625–630.
[7] Bruce, L.J., et al., 2009. The monovalent cation leak in overhydrated stomatocytic red blood cells results from amino acid substitutions in the Rh-associated glycoprotein. Blood 113, 1350–1357.
[8] Huang, C.-H., et al., 2000. Molecular biology and genetics of the Rh blood group system. Semin Hematol 37, 150–165.

Duclos Antigen

Terminology

ISBT symbol (number)	RHAG1 (030001 or 30.1)
History	Identified in 1978. Mrs Duclos made an antibody to a high-prevalence antigen that was non-reactive with Rh_{null} and U– RBCs.

Occurrence

Only one Duclos– proband has been reported.

Expression

Cord RBCs	Expressed

Molecular basis associated with Duclos antigen[1]

Amino acid	Gln106
Nucleotide	C at bp 316 in exon 2
Duclos–	Glu106 and G at bp 316

Effect of enzymes and chemicals on Duclos antigen on intact RBCs

Ficin/Papain	Resistant (enhanced)
Trypsin	Resistant (enhanced)
α-Chymotrypsin	Weakened
DTT 200 mM/50 mM	Sensitive/resistant (thus sensitive to WARM™ and ZZAP)

In vitro characteristics of alloanti-Duclos

Immunoglobulin class	IgG
Optimal technique	IAT

Clinical significance of alloanti-Duclos

No information is available because only one example of anti-Duclos has been described.

Comments

Duclos is absent from RBCs with either the U– Rh_{null} or U– Rh_{mod} phenotype.

Reference

[1] Tilley, L., et al., 2010. A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein. Vox Sang 98, 151–159.

Ol^a Antigen

Terminology

ISBT symbol (number)	RHAG2 (030002 or 30.2)
Obsolete name	Oldeide
History	Identified in 1986 by screening random donors with the multispecific serum Kirk. Named from the initials of the Ol(a+) index case whose C+c+ RBCs had a weak expression of C.

Occurrence

All populations	<0.01%

Expression

Cord RBCs	Presumed expressed

Molecular basis associated with Ol^a antigen[1]

Amino acid	Leu227
Nucleotide	T at bp 680 in exon 5
Ol(a–) (wild type)	Ser227 and C at bp 680

Effect of enzymes and chemicals on Ol^a antigen on intact RBCs

Ficin/Papain	Resistant (enhanced)
Trypsin	Resistant

In vitro characteristics of alloanti-Ol[a]

Immunoglobulin class	IgG
Optimal technique	IAT

Clinical significance of alloanti-Ol[a]

No data because antigen is rare.

Comments

Homozygosity for *RHAG*01.02* encodes an Rh_{mod} phenotype.
Ol(a+) RBCs have a weakened expression of Rh antigens (C and E) when *in trans* haplotype is informative.

Reference

[1] Tilley, L., et al., 2010. A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein. Vox Sang 98, 151–159.

DSLK Antigen

Terminology

ISBT symbol (number)	RHAG3 (030003 or 30.3)
History	Identified in 1996 and named in 2010: "DS" from "Duclos," and "LK" from "like" because the proband's RBCs were not agglutinated by the Duclos plasma.

Occurrence

Only one DSLK– proband has been reported.

Expression

Cord RBCs	Presumed expressed

Molecular basis associated with DSLK antigen[1]

Amino acid	Lys164
Nucleotide	A at bp 490 in exon 5
DSLK–	Gln164 and C at bp 490

Effect of enzymes and chemicals on DSLK antigen on intact RBCs

Ficin/Papain	Resistant (enhanced)
Trypsin	Resistant

In vitro characteristics of alloanti-DSLK

Immunoglobulin class	IgG
Optimal technique	IAT

Clinical significance of alloanti-DSLK

No data because only one example of anti-DSLK has been described.

Comments

DSLK is absent from RBCs with either the U– Rh_{null} or U– Rh_{mod} phenotype.

Reference

[1] Tilley, L., et al., 2010. A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein. Vox Sang 98, 151–159.

RHAG4 Antigen

Terminology

ISBT symbol (number)	RHAG4 (030004 or 30.4)
History	Identified in 2011, when the antibody caused severe HDFN.

Occurrence

The only antigen-positive proband was of African ancestry.

Expression

Cord RBCs	Expressed

Molecular basis associated with RHAG4 antigen[1]

Amino acid	Ile270
Nucleotide	A at bp 808 in exon 6
RHAG− (wild type)	Val270 and G at bp 808

Effect of enzymes and chemicals on RHAG4 antigen on intact RBCs

Ficin/Papain	Resistant (enhanced)
Trypsin	Resistant
α-Chymotrypsin	Presumed weakened
DTT 200 mM/50 mM	Presumed sensitive/resistant (thus sensitive to WARM™ and ZZAP)

In vitro characteristics of alloanti-RHAG4

Immunoglobulin class	IgG
Optimal technique	IAT

Clinical significance of alloanti-RHAG4

Transfusion reaction	No data because antigen is rare
HDFN	Severe[1]

Reference

[1] Poole, J., et al., 2011. A novel RHAG blood group antigen associated with severe HDFN [abstract]. Vox Sang 101 (Suppl. 1), 70.