27 Junior and Langereis Blood Group Systems

27.1 Introduction

Jr^a (JR1) and Lan (LAN1) are antigens of very high frequency in most populations. In 2012 they were shown to be located on the ATP-binding cassette transporters ABCG2 and ABCB6, respectively, and so became the sole representatives of the Junior and Langereis blood group systems.

27.2 ATP-binding cassette (ABC) transporters

ABC transporters form one of the largest and most diverse protein superfamilies and are present in all living cells and organisms. Forty-eight ABC transporters are present in humans and are located within external and internal membranes of cells. They are classified into seven subfamilies, ABCA to ABCG, based on their gene and amino acid sequences and domain organisation. A typical active ABC transporter comprises two transmembrane (TM) domains, consisting of between six and eleven membrane-spanning α-helices, and two nucleotide-binding domains (NBDs) (Figure 27.1). Half-transporters have only one TMD and one NBD, and are functionally dependent homodimer or heterodimer formation. Full transporters are usually found in the external membrane, whereas half-transporters are generally located in subcellular organelles. The NBDs, which bind and hydrolyse ATP to fuel transport activity, contain three characteristic motifs: a signature motif (LSGGQ) unique to this superfamily, flanked by Walker A and Walker B motifs.

ABC transporters translocate multifarious hydrophobic substrates across biological membranes. They have a wide variety of functions and genetic defects lead to various diseases, including cystic fibrosis. They have also been implicated in multidrug resistance in cancer. For reviews on ABC transporters, see [1–3].

27.3 Junior system, Jr^a antigen, and ABCG2

The first five examples of anti-Jr^a were described briefly in 1970 by Stroup and MacIlroy [4], who were able to test the families of four of the propositi and found a total of seven Jr(a–) siblings, none of whom had made anti-Jr^a.

27.3.1 Frequency and ethnic distribution

Jr(a–) is much less rare in Japanese than in most other populations (Table 27.1). Frequencies vary greatly in different regions of Japan with an incidence of Jr(a–) of around one in 60 in the Niigata region of northwest Japan. Jr(a–) has been found in people of Northern European extraction [9–12], particularly in Gypsy populations [12,13], in Bedouin Arabs [14], and in a Vietnamese [15].

27.3.2 Anti-Jr^a

Anti-Jr^a may be stimulated by transfusion or by pregnancy and has been detected in untransfused Jr(a–)

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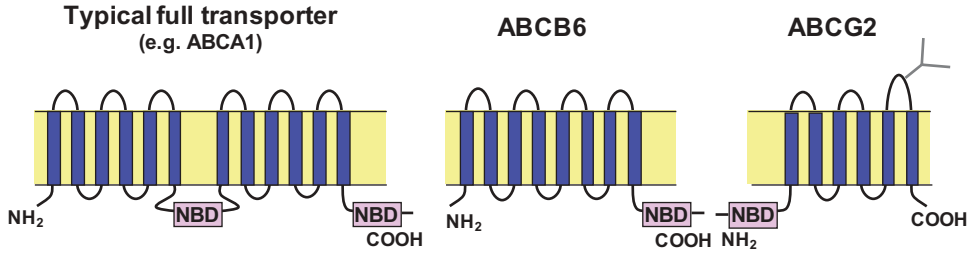

Figure 27.1 Diagram of three ABC proteins in a membrane: a typical full transporter and two half-transporters, ABCB6 (Langereis) and ABCG2 (Junior). NBD, nucleotide binding domain.

Table 27.1 Frequency of Jr^a.

Population	No. tested	No. negative	Antigen frequency	References
Japanese	19 298	5	0.9997	[5]
Japanese	28 744	19	0.9993	[6]
Japanese, Osaka	994	2	0.9980	[7]
Japanese, Niigata	460	8	0.9826	[7]
American	9545	0		[8,9]

women during their first pregnancy [16,17]. IgM anti-Jr^a was found in the sera of two Jr(a–) brothers who had not been transfused. Most anti-Jr^a are IgG and those that have been subclassed were predominantly IgG1, sometimes together with IgG3 [14,16–19]. Anti-Jr^a may fix complement [5,15,20–22].

Many transfusions of Jr(a+) red cells to patients have resulted in no signs of haemolysis and no adverse effects, although incompatible transfusion may cause a sharp rise in the titre of the anti-Jr^a, resulting in signs of an acute HTR in subsequent transfusions [21]. A patient with anti-Jr^a developed rigors after transfusion of 150 ml of crossmatch-incompatible blood [23]; another signs of a mild delayed HTR after transfusion of multiple units of Jr(a+) red cells [15]. Injection of radiolabelled Jr(a+) red cells into a patient with anti-Jr^a resulted in moderate destruction of the cells with no Jr(a+) cells remaining after 24 hours [10]. Five of 14 anti-Jr^a gave results suggesting potential clinical significance on monocyte monolayer assays [24], as did assays on some other patients [15,21]. Least incompatible red cells may be suitable for transfusion to most patients with anti-Jr^a, but Jr(a–) red cells should be selected in cases where the anti-Jr^a is of high titre.

Anti-Jr^a is a dangerous antibody in pregnancy and has been implicated in severe and fatal HDFN [11,12, 19,22], although in other pregnancies with maternal anti-Jr^a indications of HDFN have been no more than a positive DAT on cord cells or mild neonatal jaundice [5,16,17,20,25].

An IgG3 human monoclonal anti-Jr^a was produced from lymphocytes of a blood donor with anti-Jr^a [6].

27.3.3 ABCG2 and the molecular basis for Jr(a–)

In 2012 Zelinski *et al.* [26] and Saison *et al.* [27] reported, in the same issue of *Nature Genetics*, identification by very different methods that the gene encoding Jr^a is *ABCG2*. One group used SNP analysis on genomic DNA from six Jr(a–) individuals to locate a homozygous region at chromosome 4q22.1, and then identified *ABCG2* as only one of the four genes in the region expressed on red cells [26]. The other group used monoclonal anti-Jr^a to isolate a protein from cat red cells, which express the antigen strongly. The protein was then identified as an orthologue of human ABCG2 by mass spectrometry [27]. Homozygosity or compound heterozygosity for 13 different mutations in *ABCG2* were found in Jr(a–) individuals (Table 27.2). All are inactivating mutations, with the exception of a missense mutation encoding Val12Met in the N-terminal sequence before the NBD. This mutation was found in a Jr(a–) individual with anti-Jr^a, so probably prevents protein expression in the red cell membrane.

ABCG2 is located at chromosome 4q22.1, spans over 66 kb, and contains 16 exons, with the translation start site in exon 2. The promoter region has a CCAAT box, but no TATA box, and several Sp1 sites plus AP1 and AP2 sites downstream from a putative CpG island [29]. ABCG2 is a 72 kDa protein consisting of 665 amino acids.

Table 27.2 *ABCG2* mutations associated with Jr(a–) phenotype.

Nucleotide change	Location	Amino acid change	Population	References
34G>A	Exon 2	Val12Met	Asian, Caucasian	[26,28]
187–197delATATTATCGAA	Exon 2	Ile63Tyr fs 54stop	European	[27]
337C>T	Exon 4	Arg113stop	Caucasian	[28]
376C>T	Exon 4	Gln126stop	Asian, Caucasian	[26–28]
542–543insA	Exon 6	Phe182Val fs14 stop	European	[27]
706C>T	Exon 7	Arg236stop	Asian, European, European Gypsy, N. African	[26–28]
730C>T	Exon 7	Gln244stop	European	[27]
736C>T	Exon 7	Arg246stop	Caucasian	[26,28]
784G>T	Exon 7	Gly262stop	Caucasian	[28]
791–792delTT	Exon 7	leu264His fs 14stop	European Gypsy, Caribbean	[27]
875–878dupACTT	Exon 8	Phe293Leu fs 8stop	Caribbean	[27]
1111–1112delAC	Exon 9	Thr37Leu fs 20stop	Pakistani	[27]
1591C>T	Exon 13	Gln531stop	Caucasian	[28]

Jr(a–) phenotype arises from either homozygosity or compound heterozygosity.

It has a single TMD, with six membrane-spanning α-helices, an N-terminal NBD, and is glycosylated at Asn596 in the third extracellular loop (Figure 27.1). As a half-transporter, ABCG2 forms dimers or possibly oligomers in order to function (for review see [30,31]).

ABCG2 is present in many different human cells and may have multiple functions. It was first identified as a multidrug resistance protein [32]. It also functions as a high-capacity uric acid transporter, and Gln126stop, which is a cause of Jr(a–) phenotype, is considered a major cause of primary gout in Japan [33]. Val12Met, however, also responsible for Jr(a–), was not found to confer any risk. A genome-wide association study identified an association between *ABCG2* and serum uric acid levels and risk of gout in people of European and African origin [34]. Significant elevation of serum urate levels were not detected, however, in the absence of ABCG2 in pregnant Jr(a–) women [27]. Porphyrin levels were very low in the plasma and elevated in the red cells of Jr(a–) individuals, suggesting that ABCG2 may share the function of red cell porphyrin transporter with ABCB6 [27] (Section 27.4.3).

27.4 Langereis system, Lan (LAN1) antigen, and ABCB6

A severe HTR resulted in the identification of a new public antigen, Lan [35]. The patient, Mr Lan, had a Lan– brother. Two other public antigens, Gn^a and So, were later shown to be the same as Lan [36–38].

Table 27.3 Frequency of Lan in various populations.

Population	No. tested	No. negative	Antigen frequency	References
American	6653	1	0.9998	[36,38–41]
British	28 992	0	>0.9999	[42,43]
Japanese	713 384	14	>0.9999	[44]
Black South African*	6000	4	0.9993	[45]

*Including donors of mixed ethnic origin.

27.4.1 Lan antigen: frequency and variants

Screening of red cells from almost 40 000 blood donors from America, Britain, and Holland (mostly Caucasian) with alloanti-Lan revealed only two Lan-negatives (1 in 20 000) and with monoclonal anti-Lan of red cells from 713 384 Japanese donors revealed 14 Lan-negatives (1 in 50 000) (Table 27.3). Anti-Lan has been reported in two African Americans [46,47].

A quantitative variant, in which Lan is expressed very weakly, has also been shown to be inherited [48]. Red cells with this Lan-weak phenotype can easily be mistaken for

Table 27.4 *ABCB6* mutations associated with Lan– phenotype [44,56].

Nucleotide change	Location	Amino acid change	Population
197–198inG	Exon 1	Ala66Gly fs 96stop	European
574C>T	Exon 2	Arg192Trp	N. African
717G>A	Exon 3	Gln239stop	European
953–956delGTGG	Exon 4	Gly318Ala fs 8stop	European
1533–1543dupCGGCTCCCTGC	Exon 9	Leu515Pro fs 17stop	European
1690–1691delAT	Exon 11	Met564Val fs 2stop	Japanese
1709–1710delAG	Exon 11	Glu570Gly fs 21stop	European
1867delinsAACAGGTGA	Exon 14	Gly623Asn fs 3stop	European
1942C>T	Exon 14	Arg648stop	European, N. African
1985–1986delTC	Exon 15	Leu662Pro fs 15stop	European
2256 + 2t > g	Intron 16	Splicing defect	European

Lan– phenotype arises from either homozygosity or compound heterozygosity.

Lan–. Five of 15 apparent Lan– samples were subsequently shown to have weak Lan on testing with more potent reagents [49].

Cord red cells had higher Lan activity than adult cells, as determined by a monoclonal antibody [44].

27.4.2 Anti-Lan

Anti-Lan may be stimulated by transfusion or pregnancy [35,36,38,39,50,51]. There is no report of 'naturally occurring' alloanti-Lan; none of the Lan– siblings of Lan– propositi has anti-Lan. Lan alloantibodies are mostly IgG1 and IgG3, although IgG2 and IgG4 may also be present [18,24,50,52]. Some anti-Lan fix complement [35,43,50,53], others do not [39,50].

The original anti-Lan was responsible for an immediate HTR characterised by fever and chills [35]. The potential of other examples of anti-Lan to cause red cell destruction has been demonstrated by *in vivo* red cell survival studies and *in vitro* functional assays [24,53–55]. Ideally Lan– red cells should be selected for transfusion to patients with anti-Lan, although least incompatible red cells may be suitable for patients with weak examples of the antibody.

Anti-Lan has not been implicated in serious HDFN. Two babies of mothers with anti-Lan and whose cord red cells gave a positive DAT received phototherapy and one of them (whose red cells were also coated with anti-c and -Jk^a) was transfused with Lan+ red cells [41,51].

The only reported autoanti-Lan was in a patient with mild AIHA [54]. Her red cells appeared to have depressed Lan expression and gave a weakly positive DAT. Monoclonal anti-Lan was produced from lymphocytes of a donor with anti-Lan [44].

27.4.3 ABCB6 and the molecular basis of Lan–

Helias *et al.* [44] used monoclonal anti-Lan to purify the Lan antigen from red cells: an 80 kDa membrane protein identified by mass spectrometry as ABCB6. Ten inactivating mutations were identified in 11 unrelated Lan– individuals: nine were homozygous and two were compound heterozygotes (Table 27.4). Homozygosity for a missense mutation, Arg192Trp, was also responsible for a true Lan– phenotype associated with anti-Lan production [56]. Heterozygosity for three other *ABCB6* mutations (826C>T, Arg276Trp; 85–87delTTC, Phe29del; 1762G>A, Gly588Ser) appeared to cause a reduced level of Lan expression, suggesting that they are also null alleles [56].

Human *ABCB6* gene was identified by screening a human liver cDNA library with an expressed sequence tag (EST) revealed by searching a human EST database for an orthologue of a mouse ABC gene [57]. The cDNA predicted an 842 amino acid ABC half-transporter with a TMD containing eight membrane-spanning α-helices (Figure 27.1). *ABCB6* is located on chromosome 2q36, spans 11 kb, and contains 19 exons. ABCB6 exists in two forms of MW 104 kDa and 79 kDa in mitochondrial and plasma membranes [58]. During haem biosynthesis in erythroid cells, ABCB6 appears to function by importing porphyrin into mitochondria. In mature red cells, which have no mitochondria, ABCB6 may export porphyrins from the cells to prevent their accumulation [58,59].

ABCB6 may also have a general role of regulating haem biosynthesis in non-erythroid cells. Another porphyrin transporter, possibly ABCG2 (Jr^a protein, Section 27.3.3), could compensate for the absence of ABCB6 in the Lan– phenotype [44].

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