Molecular basis of the Duffy blood group system

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Abstract

ACKR1, located on chromosome 1q23.2, is the gene that encodes a glycoprotein expressing the Duffy blood group antigens. This gene is transcribed in two mRNA variants yielding two isoforms, encoding proteins with 338 and 336 amino acids. This review provides a general overview of the Duffy blood group to characterise and elucidate the genetic basis of this system. The Fy^a and Fy^b antigens are encoded by co-dominant *FY*A (FY*01)* and *FY*B (FY*02)* alleles, which differ by c.125G>A (rs12075), defining the Fy(a+b−), Fy(a−b+) and Fy(a+b+) phenotypes. The Fy(a−b−) phenotype that occurs in Africans provides an explanation for the apparent absence of *Plasmodium vivax* in this region: this phenotype arises from homozygosity for the *FY*B* allele carrying a point mutation c.1-67T>C (rs2814778), which prevents Fy^b antigen expression only in red blood cells. The same mutation has also been found on the *FY*A* allele, but it is very rare. The Fy(a−b−) phenotype in Europeans and Asians arises from mutations in the coding region of the *FY*A* or *FY*B* allele, preventing Duffy antigen expression on any cell in the body and thus are true Duffy null phenotypes. According to the International Society for Blood Transfusion, ten alleles are associated with the null expression of the Fy antigens. Furthermore, different allelic forms of *FY*B* modify Fy^b antigen expression, which may result in very weak or equivocal serology results. The mostly common found variants, c.265C>T (rs34599082) and c.298G>A (rs13962) - previously defined in combination only with the *FY*B* allele - have already been observed in the *FY*A* allele. Thus, six alleles have been recognised and associated with weak expression of the Fy antigens. Considering the importance of the Duffy blood group system in clinical medicine, additional studies via molecular biology approaches must be performed to resolve and clarify the discrepant results that are present in the erythrocyte phenotyping.

Keywords: Duffy blood group system, *ACKR1* gene, DARC, allele variants.

Introduction

The blood group systems are characterised by the presence or absence of antigens on the erythrocyte membrane, and these antigens are often polymorphic with respect to sequence and function[1]. Currently, according to the International Society for Blood Transfusion (ISBT), there are 346 erythrocyte antigens, dispersed over 36 different blood group systems[2].

The erythrocyte antigens are genetically inherited and defined by specific sequences of amino acids, constituting proteins connected to lipids or carbohydrates[3]. These antigens are important to transfusion medicine because their absence from the red blood cells of an individual can result in alloimmunisation after a transfusion with the respective antigen[1]. Among the various consequences of alloimmunisation, the following stand out: an increased risk of transfusion reactions, reduction of the number of compatible blood bags, destruction of allogeneic erythrocytes, as well as of autologous and foetal erythrocytes, in addition to damage to transplanted tissues[4].

In order to minimise the chances of an individual generating erythrocyte alloantibodies, transfusions must be phenotypically compatible to the most immunogenic antigens[5]. Although phenotyping is essential for the confirmation of the presence of alloantibodies and also for the detection of blood group antigens[6-8], phenotyping suffers from certain technical limitations because it is a subjective test, many antibodies are not commercially available and it is a labour-intensive test, so a relatively small number of donors can be typed for a relatively small number of antigens. There are also certain clinical limitations, including the difficulty of phenotyping recently transfused patients as well as red blood cells coated with IgG, and it can be challenging to distinguish an alloantibody from an autoantibody in antigen-positive people[9]. In these situations, blood group genotyping has proven to be an excellent alternative to phenotypying[10,11].

The main indications for performing such a molecular test in immunohaematology are the identification of erythrocyte antigens in recently transfused patients, in patients with a positive direct antiglobulin test (DAT+) and in situations in which there is a risk of developing haemolytic disease of the foetus and newborn (HDFN)[7,12,13]. Molecular techniques may also be used to identify the presence of variation in genes that encode blood group antigens that are expressed weakly in the membrane, thereby contributing to the prevention of possible haemolytic transfusion reactions[6].

DNA-based genotyping of the Duffy blood group system can be an important adjunct to traditional phenotyping, especially in clinical situations in which the risk of HDFN is a concern and for locating matched blood for alloimmunised patients. Accordingly, this review provides a general overview of the Duffy blood group to characterise and clarify the genetic basis of the allelic variants of this system.

Duffy blood group system

The Duffy blood group was initially reported by Cutbush in 1950, who described the reactivity of an antibody found in a male, multitransfused, haemophilic patient who had an alloantibody against an antigen, then denoted as Fy^a. This antibody was named anti-Fy^a, in honour of the patient in question[14]. A year later, an antibody was described in the serum of a multiparous woman which defined its antithetical pair, called anti-Fy^b [15]. The Duffy antigens reside in an acidic glycoprotein that spans the membrane seven times. The N-terminal portion forms the extracellular domain and the C-terminal portion forms the intracellular domain.

Biological functions

The Duffy glycoprotein, also known as the Duffy antigen receptor for chemokines (DARC), is a promiscuous receptor that binds chemokines of the C-X-C and C-C classes[16-18]. Examples of C-X-C chemokines are interleukin-8 (IL-8) and melanoma growth stimulatory activity (MGSA), while the C-C chemokines include regulated on activation, normal T expressed and secreted (RANTES) and monocyte chemotactic protein-1 (MCP-1)[16-18]. The main normal function described for DARC is that it effectively sustains homeostatic levels of circulating chemokines and modulates chemokine gradients between tissues and the blood to mediate the influx of neutrophils and monocytes from blood vessels into tissues during immune responses[19,20]. Although the specific mechanisms underlying its functions remain uncertain, there is interest in DARC as an explanatory variable for population-specific differences in disease susceptibility[21], as demonstrated by ongoing research into its role in inflammation-associated pathology and malignancy[21,22], and by the recent, though highly controversial[23], surge in interest around the antigen's role in human immunodeficiency virus infection[24].

Much of the research into this blood group has been concerned with elucidating the characteristic expression patterns among different populations[25]. Interest in the Duffy blood group rose substantially with the recognition of its role as a portal of entry for malarial parasites into human red blood cells[26]. While *Plasmodium falciparum* uses a series of receptors on the surface of human erythrocytes to invade them, *Plasmodium vivax* and *Plasmodium knowlesi* depend on an interaction with the Duffy antigen, meaning that red blood cells lacking the antigen are refractory to invasion by these merozoites[26-29]. The proportion of individuals in African populations who do not express the DARC protein in their erythrocytes is high. The gap in distribution of *P. vivax* in Africa is, therefore, viewed as the consequence of the lack of this protein on red blood cells - suggesting either an adaptive response to the disease or a selective pressure acting on the parasite[30,31]. Furthermore, a genotype-dosage effect on expression of the DARC protein has been described and the level of DARC expression is associated with susceptibility and resistance to infection[32-35]. These results imply that the red cells of heterozygotes for the silent allele bind substantially less *P. vivax* Duffy-binding protein than those of individuals with two active *FY* alleles, indicating that Duffy-negative heterozygosity confers significant protection and may have a selective advantage in areas where *P. vivax* is endemic[36]. However, since 2006 there have been reports of Duffy-negative individuals infected with *P. vivax,* both throughout Africa (Kenya, Madagascar, Mauritania, Cameroon, Angola, Equatorial Guinea, Ethiopia, and Sudan) and in Brazilian Amazon[37-46]. The mechanisms involved in this invasion remain to be elucidated: the hypotheses postulated include expansions of the copy number of gene Duffy-binding protein 1 of the *P. vivax*[47] and the use of alternative ligand-receptor pairs[30,47].

Genetic basis

ACKR1, also known as *DARC* or *FY* (NCBI), is the gene that encodes a transmembranous glycoprotein expressing the Duffy blood group antigens. Its genetic locus was reported to be on chromosome 1, located formerly in the region 1q21-25 by linkage analysis[48]. Later, the position of the gene was refined to 1q23.2. The gene (sequence NC_000001.11, region: 159204013-159206500) is transcribed as two mRNA variants. Chaudhuri *et al*. reported that the first mRNA variant has one exon[49]. Subsequently, Iwamoto *et al.* demonstrated the existence of a spliced mRNA variant that has two exons with the intron encompassing sequences in the initial part of the first mRNA variant[50]. Despite encoding for a shorter protein, the second mRNA variant has a longer transcript than the first because of a longer 5′ untranslated region. These two distinct transcript isoforms are expressed from separate promoters, yielding distinct protein products. The major transcript is derived from exon 1 and exon 2 of *ACKR1*; the minor product is a transcript initiated at the beginning of exon 2. The minor and major transcripts generate, respectively, isoform A (NM_001122951.2/ NP_001116423.1), encoding a protein of 338 amino

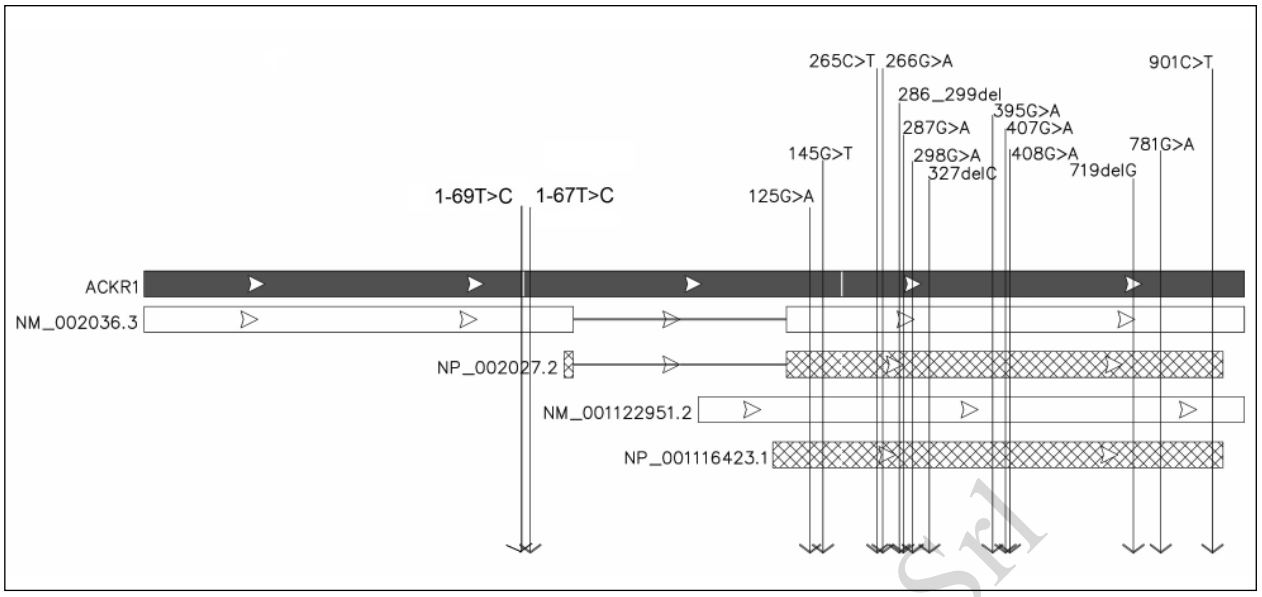

Figure 1 - *ACKR1* gene structure and proteins and mRNA isoforms.
Viewing the figure from top to bottom: the black box represents the *ACKR1* gene, the white box shows the mRNA of isoform B (NM_002036.3) and the dashed box shows the isoform B protein (NP_002027.2); the next white box represents the mRNA of isoform A (NM_001122951.2) and the dashed box shows the protein of isoform A (NP_001116423.1). The arrows indicate the positions of the main genetic variants already described in this gene.

acids and isoform B (NM_002036.3/NP_002027.2), which encodes a protein of 336 amino acids[51] (Figure 1).

The nucleotide and amino acid sequences of *ACKR1* were renumbered after the discovery that the spliced mRNA is the major product of the gene. It was proposed that the first nucleotide of the translation initiation codon of the major spliced mRNA be numbered nucleotide 1. This numbering convention avoids inconsistencies created by differing lengths of the 5-prime untranslated region arising from alternative transcription initiation sites[52]. This isoform B has been chosen as the "canonical" sequence that is known to be relevant for blood group genotyping because is expressed in erythroid lineage cells. All positional information in this review refers to isoform B.

The antithetical antigens, Fy^a and Fy^b, are encoded by co-dominant *FY*A (FY*01)* and *FY*B (FY*02)* alleles, which differ by a single nucleotide polymorphism c.125G>A (rs12075)[49,50]. On the *FY*A* allele, the base is guanine (G), and on the *FY*B* allele the base is adenine (A). This missense mutation produces a codon for glycine in the *FY*A* allele and a codon for aspartic acid in the *FY*B* allele at position 42 of the major product (p.Gly42Asp)[53-55], defining the Fy(a+b−), Fy(a−b+) and Fy(a+b+) phenotypes. Additionally, a number of variants have been identified that cause weak ($+^w$ or **W*) and null (0 or **N*) expression of Duffy Fy^a or Fy^b antigens (Table I). According to the ISBT, there are two mutations associated with weak expression of Fy^a and five mutations associated with weak expression of Fy^b. Seven mutations that cause the null expression of Fy^a have already been observed and three such mutations for Fy^b have been found. Despite the high genetic variability related to Duffy antigen production, some of these variants are more commonly associated with null or weak expression of these antigens than others, and they are described below.

The Fy(a−b−) phenotype, also known as "erythrocyte silent" (Fy^{es}), occurs in African lineages and, depending on the region, has a prevalence of nearly 100% (e.g. in West Africa) and is also found at greater than 80% frequency in African Americans[26,56]. This phenotype arises from homozygosity for an *FY*B* allele carrying a point mutation c.1-67T>C (rs2814778) in the 5' untranslated region[57]. This mutation gives rise to the $FY*B^{ES}$ (*FY*02N.01*) allele, which impairs promoter activity in erythroid cells by disrupting a binding site for the GATA-1 erythroid transcription factor[57]. This mutation prevents Fy^b antigen expression only in red blood cells but not in other tissues[53,58]. As a result, Africans with the Fy(a−b−) phenotype rarely make anti-Fy^b[50,59,60]. The same mutation (previously described as c.-33T>C and c.-46T>C) has been found on the $FY*A^{ES}$ allele (*FY*01N.01*), but only in a heterozygous state in inhabitants of Papua New Guinea and Sudan; it is very rare[35,61]. Písacka *et al.* reported a novel mutation at position c.1-69 in the *FY* promoter that also disrupts the GATA motif and correlates with silencing of the *FY*A* allele, causing a Fy null phenotype[62].

The few documented cases of the Fy(a−b−)

Table I – Variants of the Duffy blood group system.

Fy expression	Allele name	Nucleotide	Region	Amino acid	dbSNP
Fy(a+)	*FY*01*	c.125G	Exon 2	p.Gly42	rs12075
Fy(b+)	*FY*02*	c.125G>A	Exon 2	p.Gly42Asp	rs12075
		Null alleles			
Fy(a) null					
Fy(a−) erythroid cells only	*FY*01N.01*	c.1-67T>C	5'UTR	p.0	rs2814778
Fy(a−)	*FY*01N.02*	c.286_299del	Exon 2	p.Trp96Thrfs	rs587776507
Fy(a−)	*FY*01N.03*	c.408G>A	Exon 2	p.Trp136Ter	–
Fy(a−)	*FY*01N.04*	c.287G>A	Exon 2	p.Trp96Ter	rs750052723
Fy(a−)	*FY*01N.05*	c.327delC	Exon 2	p.Phe109fs	–
Fy(a−)	*FY*01N.06*	c.395G>A	Exon 2	p.Gly132Asp	rs530992295
Fy(a−)	*FY*01N.07*	c.719delG	Exon 2	p.Gly240fs	–
Fy(a−)	–	c.1-69T>C	5'UTR	p.0	–
Fy(b) null					
Fy(b−) erythroid cells only, Fy^{es}	*FY*02N.01*	c.1-67T>C	5'UTR	p.0	rs2814778
Fy(b−)	*FY*02N.02*	c.407G>A	Exon 2	p.Trp136Ter	rs76819093
Fy(b−)	*FY*02N.03*	c.781G>A	Exon 2	p.Gly261Arg	–
		Weak alleles			
Fy(a) weak					
$Fy(a+^{w})$	*FY*01W.01*	c.265C>T	Exon 2	p.Arg89Cys	rs34599082
$Fy(a+^{w})$	*FY*01W.02*	c.265C>T, 298G>A	Exon 2	p.Arg89Cys, Ala100Thr	rs34599082, rs13962
Fy(b) weak					
$Fy(b+^{w})$, Fy^{x}	*FY*02W.01*	c.265C>T, 298G>A	Exon 2	p.Arg89Cys, Ala100Thr	rs34599082, rs13962
$Fy(b+^{w})$, Fy^{x}	*FY*02W.02*	c.145G>T, c.265C>T, 298G>A	Exon 2	p.Ala49Ser, Arg89Cys, Ala100Thr	– rs34599082, rs13962
$Fy(b+^{w})$	*FY*02W.03*	c.266G>A	Exon 2	p.Arg89His	rs371909350
$Fy(b+^{w})$	*FY*02W.04*	c.901C>T	Exon 2	p.Pro301Ser	rs753831902

The nucleotide position is based on NCBI data. fs: frameshift; UTR: untranslated region.

phenotype in Europeans and Asians arise from mutations in the coding region of the *FY*A* or *FY*B* allele[55,63-65]. These mutations, when present in the homozygous state, prevent Duffy antigen expression on any cell in the body and thus are true Duffy null phenotypes. Consequently, these individuals are at risk of being alloimmunised when exposed to red blood cells expressing Fy antigens[66].

Different allelic forms of the Duffy blood group gene modify the antigen's expression level, which may cause problems in blood group phenotyping, leading, specifically, to very weak or equivocal serology typing results[66-69]. The most common variants, c.265C>T (rs34599082) and c.298G>A (rs13962), cause a substitution of arginine to cysteine at position 89 (p.Arg89Cys) and an alanine to threonine substitution at amino acid 100 (p.Ala100Thr) of glycoprotein Duffy, respectively. The aforementioned two variants usually result in the weak expression of Fy^{b} (*FY*02W.01* allele, also referred to as Fy^{x} antigen). This allele has been described mainly among Europeans, with a frequency varying from 2 to 3,5%; but it has not been found in Africans[69-73]. The c.298G>A variant alone does not result in reduced Fy^{b} expression[70]. Another mutation linked to weak expression of the antigen Fy^{b}, c.145G>T, changes the amino acid alanine to serine at position 49 (p.Ala49Ser), generating the *FY*02W.02* allele[69].

Although less common, weak serological reactivity of the Fy^{a} antigen has already been observed and Lopez *et al.* recently investigated this phenotype. They identified two variants of the *FY*A* allele, 265T and 298A, which were consistently present in the donor. Prior to this study, these variants had only been defined in combination with the *FY*B* allele. Reflecting these facts, *FY*01W.02* was the provisional name given to the new allele by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology[74]. One potential explanation for such complexity among Fy antigens could be the co-expression of alternative *ACKR1* gene product

Table II - Phenotype and genotype correlations of the main polymorphisms.

Phenotype	c.125G>A[1]	c.265C>T[2]	c.298G>A[3]	c.1-67T>C[4]	Predicted genotype	Predicted antigen
Fy(a+b−)	G/G	C/C	G/G	T/T	*FY*A/FY*A or FY*01/FY*01*	Fy^a
	G/A	C/C	G/G	T/C	*FY*01/FY*02N.01*	Fy^a/Fy^{es}
Fy(a+b+)	G/A	C/C	G/G	T/T	*FY*A/FY*B or FY*01/FY*02*	Fy^a/Fy^b
	G/A	C/T	G/A	T/T	*FY*01/FY*02W.01*	Fy^a/Fy^x
Fy(a−b+)	A/A	C/C	G/G	T/T	*FY*B/FY*B or FY*02/FY*02*	Fy^b
	A/A	C/T	G/A	T/T	*FY*02/FY*02W.01*	Fy^b/Fy^x
	A/A	T/T	A/A	T/T	*FY*02W.01/FY*02W.01*	Fy^x
	A/A	C/T	G/A	T/C	*FY*02W.01/FY*02N.01*	Fy^x/Fy^{es}
	A/A	C/C	G/G	T/C	*FY*02/FY*02N.01*	Fy^b/Fy^{es}
Fy(a−b−)	A/A	C/C	G/G	C/C	*FY*02N.01/FY*02N.01*	Fy^{es}/Fy^{es}

[1]rs12075 predicts the expression of the Fy^a and Fy^b antigens; [2]rs34599082 determines weak expression of the Fy^b antigen; [3]rs13962 determines weak expression of the Fy^b antigen; [4]rs2814778 prevents expression of Fy^b antigen in red blood cells.

Table III - Allele frequencies according to the 1000 Genomes Project.

dbSNP allele	AFR[1]	AMR[2]	EAS[3]	SAS[4]	EUR[5]
rs12075*G Fy(a+)	0.019	0.461	0.923	0.640	0.398
rs12075*A Fy(b+)	0.981	0.539	0.077	0.360	0.602
rs34599082*T $Fy(b+^w)$ or $Fy(a+^w)$	0.000	0.007	0.001	0.004	0.013
rs13962*A $Fy(b+^w)$ or $Fy(a+^w)$	0.005	0.094	0.000	0.091	0.184
rs530992295*A $Fy(a^{null})$	0.000	0.000	0.000	0.002	0.000
rs2814778*C $Fy(b^{null})$ or $Fy(a^{null})$	0.964	0.078	0.000	0.000	0.006

[1]AFR: African (from Yoruba in Ibadan, Nigeria; Luhya in Webuye, Kenya; Gambian in Western Divisions in the Gambia; Mende in Sierra Leone; Esan in Nigeria; Americans of African ancestry in the SW USA; African Caribbeans in Barbados); [2]AMR: Admixed Americans (Mexican ancestry from Los Angeles, USA; Puerto Ricans from Puerto Rico; Colombians from Medellin, Colombia; Peruvians from Lima, Peru; [3]EAS: East Asian (Han Chinese in Beijing, China; Japanese in Tokyo, Japan; Southern Han Chinese; Chinese Dai in Xishuangbanna, China; Kinh in Ho Chi Minh City, Vietnam); [4]SAS: South Asian (Gujarati Indians from Houston, Texas, USA; Punjabi from Lahore, Pakistan; Bengali from Bangladesh; Sri Lankan Tamils from the UK; Indian Telugu from the UK); [5]EUR: European (Utah residents with northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain; Tuscany in Italy; Finnish in Finland; British in England and Scotland; Iberian population in Spain).

Table IV - Typical Duffy phenotype frequencies.

Phenotype	Frequencies (%)		
	Europeans	*Africans*	*Asians*
Fy(a+b−)	20	10	89,2
Fy(a−b+)	32	20	1,8
Fy(a+b+)	48	3	9,0
Fy(a−b−)	Rare	67	0

Data compiled from Mourant et al. and De Silva *et al.*[78,79].

isoforms and distinct post-translational modifications between the isoforms acting as immunogens[51]. The phenotypic and genotypic correlations of the main polymorphisms are presented in Table II.

Geographic distribution

It is characteristic of this blood group system that there is a great diversity of distributions of the Duffy antigenic determinants among different ethnic groups. Some of these variants were described in the 1000 Genomes Project database. According to these data, the rs12075*A single nucleotide polymorphism, which determines the *FY*B* ancestral allele (0.541), is more prevalent globally than the rs12075*G variant, which determines the *FY*A* allele (0.459). The *FY*B* allele is common in Africans (0.981), but not in East Asians (0.077). On the other hand, the *FY*A* allele is dominant in East Asians (0.923), but is infrequent in Africans (0.019). Finally, the allele frequencies of the variants that determine the Fy^x and Fy^{es} antigens are highest in Europeans and Africans, respectively (Table III).

The Duffy system is considered one of the most attractive chromosomal loci for evaluating the impact of natural selection in different geographical regions[75,76]. Because the mutation that confers protection from infection by *P. vivax* prevents the expression of the DARC protein only in erythrocytes, it is possible to observe differences in phenotypic and genotypic frequencies of the Fy^b antigen in Africans. Although the most common genotype is *FY*B/FY*B*, almost all of the samples type serologically as Fy(a−b−)[77]. Table IV presents possible phenotypes among different populations.

Clinical significance

The anti-Fy^a antibody is found mainly following transfusion and, less frequently, as a result of pregnancy; it is almost never naturally occuring[80,81].The anti-Fy^b

is about 20 times less common than the anti-Fy^a and is generally present in sera in combination with other antibodies[82].These antibodies are predominantly of the IgG1 type subclass, with lesser contributions from other subclasses, for example, IgG2 (18%) and IgM (25%)[83,84]. Both antibodies cause immediate and delayed haemolytic transfusion reactions[1,85].

The Fy^a and Fy^b antigens are expressed in erythroid and non-erythroid cells, such as endothelial cells, and also in epithelial cells in various organs, including the brain, kidneys, spleen, heart, lungs, pancreas and placenta[86] - lending this system an important role in the inflammatory response, in allograft rejection and, possibly, in histocompatibility. The Duffy blood group is thus a polymorphic system that poses a major challenge for researchers at phenotypic, genotypic and tissue levels[87].

Conclusions

In view of the importance of the Duffy blood group system in clinical medicine, further studies utilising molecular biology approaches must be developed for the purpose of elucidating and characterising new sequence variants. Such molecular typing can help resolve and clarify the equivocal and discrepant results that arise in erythrocyte phenotyping performed by haemagglutination assays. These techniques, used together, contribute to the optimal use of blood units and, therefore, to the quality of transfusion practice.

The Authors declare no conflicts of interest.

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