

Transfusion Medicine and Molecular Genetic Methods

Abstract

Transfusion procedures are always complicated by potential genetic mismatching between donor and recipient. Compatibility is determined by several major antigens, such as the ABO and Rhesus blood groups. Matching for other blood groups (Kell, Kidd, Duffy, and MNS), human platelet antigens, and human leukocyte antigens (HLAs) also contributes toward the successful transfusion outcomes, especially in multitransfused or highly immunized patients. All these antigens of tissue identity are highly polymorphic and thus present great challenges for finding suitable donors for transfusion patients. The ABO blood group and HLA markers are also the determinants of transplant compatibility, and mismatched antigens will cause graft rejection or graft-versus-host disease. Thus, a single and comprehensive registry covering all of the significant transfusion and transplantation antigens is expected to become an important tool in providing an efficient service capable of delivering safe blood and quickly locating matching organs/stem cells. This review article is intended as an accessible guide for physicians who care for transfusion-dependent patients. In particular, it serves to introduce the new molecular screening methods together with the biology of these systems, which underlies the tests.

Keywords: Blood groups, genetic marker, human platelet antigen and human leukocyte antigen, transfusion

Introduction

Transfusion is an important medical treatment, especially in trauma cases. In the following sections, we discuss the critical aspects of transfusion medicine. These include the pretransfusion procedures, molecular structures of genes encoding clinically relevant transfusion antigens, and several techniques for blood group, human platelet antigen (HPA), and human leukocyte antigen (HLA) typing (i.e., serological matching and molecular platforms such as polymerase chain reaction (PCR)-sequence-specific primer (SSP), PCR-sequence-specific oligonucleotide (SSO) using Luminex, and sequence-based typing [SBT]). We conclude by considering future prospects for transfusion medicine (e.g., donor recruitment strategy). Emphasis is given to the molecular bases of blood groups, HPA, and HLA because they are the primary targets of immune responses and stand as major barriers to transfusion success. Alloantibodies against these antigens may develop before transfusion in sensitized patients due to previous transfusion and/or pregnancy. Their presence creates

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another barrier for these important medical procedures. Nonetheless, the adverse effects of these alloantibodies only appear if there are genetic disparities between donors and recipients in genes coding for these clinically relevant transfusion markers.

Transfusion Medicine

Blood transfusion has been practiced throughout human history from Egyptian antiquity through Ancient Rome and forward to the modern era of hematology. Blood has always been viewed as a sacred entity. Medieval societies regarded blood as an analog to food such that this substance was customarily ingested (rather than being transfused) as part of their religious rituals for rejuvenation, to maintain a youthful life and to mythically “calm the unrestful soul.”^[1,2] In modern medicine, blood transfusion is one the most common therapies used for saving life as a result of severe bleeding and/or anemia.^[3-5]

Pretransfusion procedures

Contemporary blood transfusion practices can generally be regarded as safe although zero risk is almost impossible to achieve. With >90 million of donations annually,^[6] it is imperative that all blood services

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should put in place an effective screening procedures to safeguard their blood products but at the same time allow them to maintain sufficient supplies. As such pretransfusion precautionary measures which include screening of potential donors, deferral systems, screening donated blood for transfusion-transmitted infection (TTI), and safe storage of whole blood and blood products, antibody screening and identification and compatibility testing have to be implemented.^[7]

Donor screening and deferral

Donor guideline criteria are almost standard across the world. Ideally, blood donations must be made on a voluntary basis, with no financial incentive. In many developed countries, 100% voluntary donation has been achieved,^[8] but in most developing and transitional states, contributions are still largely dependent on paid donors or come from family members and relatives.^[9-11] Donor guideline criteria cover aspects of life history and lifestyle, which include age, current and past health conditions such as type of illness, medication, surgical procedure, and transfusion, travel history, and physical examination by measurement of hemoglobin (Hb) and blood pressure level. The minimum Hb level requirement is at least 120 g/L for women and 130 g/L for men.

Potential donors are required to fill in a questionnaire detailing all these aspects. A short interview will be carried out on all potential blood donors to clarify any doubtful matters before donation.^[12] The questionnaire and interview aim to identify and exclude prospective high-risk individuals from donating. It is imperative to avoid any sources that might contaminate the blood supply with any infectious pathogens or certain medications and drugs that may threaten a recipient's health. Likewise, these procedures are designed to protect potential donors' health and well-being, for example, by excluding underweight or anemic persons. Once high-risk individuals are identified, they will be prohibited from donating blood or blood components depending on the cause of deferral. This embargo can be either permanent or temporary. The highest risk group contains those individuals who are positive for human immunodeficiency virus (HIV), syphilis, hepatitis B, and hepatitis C and are sex workers. All such persons are deferred permanently from donating blood or any blood components. Individuals who are going through an abortion, tattooing, or recovering from minor or major surgery are prevented from making donations for at least 6 months. Contagious diseases such as malaria, meningitis, pneumonia, and typhoid also trigger the same deferral period. Following immunization, the deferral interval can be as short as 48 h to as long as 1 year depending on type.^[7]

Trying to maximize the safety of blood products means that the loss of both donors and blood donations becomes inevitable. The National Blood Service UK reported

losing 60,000 units of blood annually due to their donor deferral policy.^[13] Worse, it is also reported that donors who are temporarily deferred are unlikely to come back for subsequent donations.^[14-16] Moreover, recruiting new donors also incurs an extra cost for blood services, estimated at glucose binding protein 20–30/donor in the UK.^[13] It is essential for blood services to try to balance their strict criteria to ensure public safety while trying to minimize the loss of both blood donors and blood collections.

In Malaysia, as currently practiced, all qualified donors who may be able to donate whole blood should not be <8 weeks provided Hb level >12.5 g/dL and not <2 weeks for plasma or platelet apheresis donation with a maximum of 15 L/year.^[7] Donations of whole blood, typically of 450 ml, can be separated into red cell concentrate, platelets, and freshly frozen plasma. Cryoprecipitate can be prepared from fresh frozen plasma, while buffy coat is extracted from the leukocyte component if requested. Other blood derivatives include albumin, coagulation concentrates (factor VIII, von Willebrand factor), and immunoglobulin, all of which can be fractionated from plasma.^[17,18]

The prevalence of TTIs is low,^[19] but these cases frequently cause fatal outcomes.^[20-22] Hence, there is a legal and statutory responsibility for blood services to ensure that the blood products that they supply are safe and do not transmit blood-borne pathogens to recipients. Therefore, in Malaysia, all blood and blood components must be tested for the presence of any transmissible infectious agents such as syphilis, hepatitis viruses (hepatitis B virus and hepatitis C virus), and HIV.^[7] In addition to this mandatory screening, blood services may also carry out additional screening as deemed necessary to maximize blood safety. Chosen tests may depend on the local population, epidemiological conditions, and geographical area (e.g., malaria, Chagas disease, human T-cell lymphotropic viruses I/II, and human cytomegalovirus). Some discretionary screening includes checking for any known or emerging pathogens that are transmissible by blood include variant Creutzfeldt–Jakob disease, babesiosis, dengue, chikungunya, and West Nile virus.^[23] Therefore, a system should be in place to ensure that all blood released into stock have been tested negative for TTI.^[24-26]

Compatibility testing

Compatibility testing involves ABO and Rh blood group typing, antibody screening, and identification plus cross-matching procedures. All pretransfusion blood must be identified according to ABO type and Rh status by full grouping procedure (forward and reverse methods). Patient's serum is also screened for the presence of any unexpected or clinically relevant antibodies to red cells before the blood can be issued.^[7] Additional screening for other antibodies can be done based on the blood group profile in one population.^[27]

In the cross-matching procedure, a patient's serum is tested directly for compatibility with donor red cells. Oftentimes, cross-match testing is performed minimally using an antiglobulin phase when the patient has a positive screening test or has a previous record of clinically significant antibody. However, with the advance of technology, the cross-matching is no longer restricted to manual serological technique but has been expanded to include automated testing as well as nonserological molecular techniques.^[28,29] Any positive antibodies screening must be further investigated. If antibody screening test is positive and/or incompatible cross-match detected, antibody identification should be performed before transfusion using a reagent red cell panel that covers all the significant antigens. Once the antibody is identified, blood should be selected which is negative for the relevant antigen.^[7]

In certain cases, such as in multitransfused patients, other blood group antigens (e.g., Kell, Kidd, and MNS) as well as HPA and HLA are typed to ensure compatibility between donor and recipient. In practice, blood group antigen is important for packed/red cell transfusion while HPA and HLA are essential for platelet transfusion.^[30-32] We would like to highlight that HLA is also crucial in the transplantation setting, which does not fall within the scope of the present review; however, the review by Edinur *et al.*^[33] provided more details regarding this. The molecular bases of blood groups, HPA, and HLA and their typing platforms are discussed in the following sections; on genetic markers and genotyping of clinically relevant transfusion markers.

Adverse effects of transfusion

Despite stringent donor selection criteria and meticulous pretransfusion testing, all transfusions still carry some degree of risk. A transfusion reaction can be defined as any unfavorable response occurring in a patient during or following receipt of any whole blood or blood products by transfusion. Adverse reactions can be caused by human error at any stage of the pretransfusion procedures.^[34-36] Even without any mistakes, there is still a risk of developing natural undesirable effects of transfusion. Alloimmunization is frequently observed in multiply transfused patients or as a result of pregnancy.^[37-39]

Apart from infectious causes, the adverse effects of transfusion can be grouped into immune and nonimmune and further subdivided into immediate or delayed reactions. Immediate reaction usually occurs within 48 h, while a delayed reaction may take days to years to develop following transfusion. Febrile nonhemolytic transfusion reactions, acute hemolytic transfusion reaction (HTR), allergic reaction, and anaphylactic reactions as well as transfusion-related acute lung injury all come under the category of immediate and immune-related reactions. Complications such as transfusion-associated graft-versus-host disease, posttransfusion purpura (PTP),

alloimmunization, and immunosuppression are considered to be part of the delayed and immune-mediated category. For example, immune-mediated acute HTR is due to ABO incompatibility between donor and recipient, which leads to intravascular or extravascular hemolysis of red cells.^[40] In addition, it can be caused by unexpected antibodies to the other blood group antigens. The incidence of HTR is estimated to be 1:70,000 of units transfused.^[41] Malhotra *et al.* reported a case of a patient who developed acute HTR only after receiving a few milliliters (ml) of a blood transfusion. Further investigation revealed that this patient had received a mismatched blood unit. The patient was initially mistyped as group "O" instead of their actual "Bombay" blood group phenotype due to the discrepancies and imprecise interpretation of the serological results.^[42] A similar case was reported earlier, but this time, the adverse reaction was caused by multiple alloantibodies. Here, a Korean patient suffered two consecutive episodes of acute HTR due to multiple antibodies including anti-E, anti-C, and anti-JKb.^[43] The lesson to be learned from this second case is the importance of antibody screening tests during pretransfusion testing. Already standard in most countries, this test is being implemented as part of the routine procedure in the pretransfusion screening (provided in "Compatibility Testing" section). Perhaps, this type of screening should be made mandatory everywhere, particularly for vulnerable group of patients, i.e., in multitransfused patients, pregnant women, and preoperative patients, to prevent complications arising from blood transfusions. Another example of adverse reaction due to blood transfusion is PTP, which is rare, but can be life-threatening. It is defined as thrombocytopenia that occurs 5–7 days following red cell or platelet transfusion.^[40] Its prevalence varies from 1:50,000 to 100,000 transfusions and most typically occurs in the middle-aged and older women.^[44] PTP is caused by alloimmunization to HPA, most commonly due to HPA-1a, although HPA-1b, HPA-3a, HPA-3b, and HPA-4b have also been documented as causal, either as singly or in combinations.^[45] For instance, Rafei *et al.* reported a case concerning a middle-aged multiparous woman who developed PTP a week after receiving a packed cell transfusion. The culprit in this incident was identified as HPA-1a antibodies.^[46] Likewise, the literature also contains documented cases of PTP as the result of HPA-1b antibodies, although the frequency of such cases are quite low.^[47,48] Iron overload and air embolisms are two examples of delayed nonmediated reactions, while transfusion-associated circulatory overload and damaged erythrocytes can be observed immediately due to nonimmune factors.^[49,50]

Genetic Markers

Transfusion of blood/blood components is always restricted and complicated by incompatibility between donor and recipient, especially in multitransfused and

transfusion-dependent patients. This is determined by several antigens such as blood groups and HPA which acting as a marker of tissue identity. For a routine transfusion, only ABO and Rhesus need to be matched, but this may be extended to include other clinically relevant blood groups (Kell, Kidd, Duffy, and MNS), HPA, and HLA for highly immunized and multitransfused patients.^[30,32,38] These antigens are encoded by highly polymorphic genes and thus present a challenge for finding suitable donors for particular patients. Incompatible antigens become targets for the recipient's immune system. Hence, high-quality donor-and-recipient matching has become clinically and practically important in ensuring successful transfusion procedures. Several markers of tissue identity important in transfusion are discussed in the following subsections.

Blood groups

Our knowledge about blood groups has evolved at rapid pace since their first discovery early in the 20th century.^[51] At the time of writing, there are no less than 33 blood systems recognized by the International Society of Blood Transfusion, with two more pending approval. To name but a few, these systems include the H antigen, Rhesus, MNS, Lutheran, Kell, Duffy, and Kidd blood groups, with a total of more than 300 antigens between them.^[52] The ABO system plays the most clinically significant role in transfusion followed by the highly complex Rh system.

Blood groups are found to be population specific and can be used as markers for ethnicity. For examples, the Diego antigen, Di^a has high prevalence among people of Mongoloid origin but rarely found in other populations.^[53,54] The JS^a antigen from the Kell blood group belongs almost exclusively to those of African

descent,^[55] while the Kidd phenotype, JK (a+b+) occurs frequently in Asians and Caucasians.^[56] The Duffy blood system which consists of two antigens, Fy^a and Fy^b, varies enormously across the world. The Fy (a-b-) phenotype of the Duffy blood group system is most often seen in Black populations with a general consensus that this phenotype confers some protection against invasion by the malaria parasites *Plasmodium vivax* and *Plasmodium knowlesi*.^[57-60] A recently approved blood group, the JR antigens, namely the JR (a-) phenotype is noted to be highest in Japanese populations, particularly in Niigata individuals, but also occurs with low frequency in Europeans.^[61-63] The following blood group systems have been extensively characterized at the population level^[64,65] [Table 1 and the following subsections provides more information].

ABO blood group system

The precursor for the ABO blood group is the H antigen protein, encoded by the H gene on chromosome 19. The H antigen is converted to A or B antigens by α 1,3-N-acetylgalactosaminyltransferase and α 1,3-D-galactosyltransferase, respectively, encoded by the ABO gene complex on chromosome 9. In contrast, the O allele contains a deletion (Δ 261G) in exon 6 of the B allele resulting in loss of enzymatic activity and H antigen remains unchanged.^[64,67] Together, combinations of A, B, and O alleles produce six genotypes (AA, AO, BB, BO, AB, and OO) and four phenotypes; A, B, AB, and O. In addition, inactivating mutations in H gene produce the very rare autosomal recessive phenotype (O_h or Bombay phenotype) and carriers develop isoantibodies toward A, B, and H antigens.^[68] Individual with Bombay phenotype cannot accept transfusion even from the otherwise "universal" donor with a blood type O.

Table 1: List of blood groups described in this report and their molecular bases

System	Chromosome	Protein	Polymorphism	Molecular basis	Amino acid change
ABO	9	Transferase	A→B	C526G, G703A, C796A, G803C	R176G, G235S, L266M, G268A
			A→O	Δ 261G	Truncated glycosyltransferase
MNS	4	TM	M→N	C59T, G71A, T72G	S20L, G24E
		TM	s→S	C143T	T48M
Rhesus	1	TM	D→variant D	Deletion/hybrid/SNPs	Partial, weak and D-negative
		TM	C→c	C48G, A178C, G203A, T307C	C16W, I60L, S68N, S103P
		TM	e→E	G676C	A226P
Lutheran	19	TM	Lu ^a →Lu ^b	G230A	R77H
Kell	7	TM	k→K	C578T	T193M
Duffy	1	TM	Fy ^a →Fy ^b	G125A	G42D
		-	Fy ^b →Fy	T-67C (GATA-1)	Noncoding
Kidd	18	TM	Jk ^a →Jk ^b	G838A	D280N
Diego	17	TM	Di ^a →Di ^b	C2561T	P854L
Dombrock	12	GPI	Do ^b →Do ^a	G793G	D265N
Colton	7	TM	Co ^a →Co ^b	C134T	A45V

GPI: Glycol proteins anchored to the membrane by a glycosylphosphatidylinositol tail, TM=Transmembrane proteins

Rhesus blood group system

The Rh blood group is clinically important in blood transfusion and gestation.^[69] The system consists of five antigens; D, C, c, E, and e. The D antigen is encoded by RHD gene while the other antigens are encoded by the RHCE gene [Table 1]. These two genes are closely linked and located on chromosome 1p34.3-p36.1.^[70] Individuals will either be D-positive or D-negative (aka Rhesus-positive and -negative, respectively) no matter which RHCE antigens they carry. The D-negative phenotype occurs due to mutation in the RHD gene as well as by genetic exchange between the highly homologous RHD and RHCE genes.^[71,72] The same mechanisms have also generated other variants of the D antigen such as “weak D” and “partial D,” which are found at low incidence in the world populations.^[73] The most commonly occurring Rhesus phenotypes are DCcee in Whites, Dccee in Blacks, and DCCee in Asians.^[74]

MNS blood group system

The MNS antigens are integral membrane proteins encoded by two homologous members of the glycoporphin (GYP) gene family, GYPA and GYPB, located on long arm of chromosome 4.^[75,76] The GYPA gene codes for M and N blood group antigens, while GYPB gene codes for the S and s blood group antigens. These two pairs of polymorphic antithetical and codominant antigens differ by three (C59T, G71A, T72G) and one (C143T) single nucleotide substitutions, respectively [Table 1]. Further, deletions, misalignment during meiosis, and gene conversion between the closely linked and homologous GYPA and GYPB genes give rise to low incidence of MNS variants such as En (a-), S-s-U-, M^k and Miltenberger.^[77,78]

Kell blood group system

The Kell blood group antigens are 93-kDa type II glycoproteins (GPs) that are linked by a single disulfide bond to an integral membrane protein, XK.^[79] The Kell and XK proteins are encoded by separate genes KELL and XK located on chromosomes 7q33 and Xp21.1, respectively.^[56] There are epistatic interactions between these two gene products as the absence of the KX protein will reduce expression of the Kell antigens (e.g., as in McLeod syndrome; Daniels^[80]). The weak expression of the Kell antigens can also be seen in the K_{mod} red blood cell (RBC) phenotype which is associated with a missense mutation (1208G>A) in exon 10 of the Kell blood group gene.^[81]

Overall, the Kell blood group system comprises >34 serologically defined variants across three sets of antigens; K and k; Kp^a, Kp^b, and Kp^c; and Js^a and Js^b.^[82] The K and its antithetical *k* allele only differ by a T578C substitution which causes a Met193Thr amino acid replacement.^[83] The Kp^b allele is the most common in all populations and only differs from Kp^a and Kp^c by

a single amino acid substitution at codon 281 of exon 8. The Kp^a codon codes for tryptophan (TGG), Kp^b for arginine (CGG), and Kp^c for glutamine (CAG). Equally, a Pro597Leu substitution differentiates between the Js^a and Js^b alleles, respectively.^[72,84]

Kidd blood group system

The JK or SLC14A1 (solute carrier family 14, member 1) gene located on chromosome 18q11-q12 codes for the Kidd antigens. Variants at this locus, the JKA and JKB alleles, code for the two codominant Kidd antigens, Jka and Jkb, respectively.^[80] The JKA/JKB polymorphism involves a single nucleotide transition (G838A) that produces the three phenotypes: Jk (a + b-), Jk (a - b+), and Jk (a+b+).^[85]

The occurrence of the Jk_{null} phenotype or Jk (a-b-) has been observed in Polynesian and Finnish populations.^[86] The Jk_{null} phenotype occurs due to mutations in either the 3'-acceptor splice site of intron 5 (IVS5-1G>A) or the 5-splice site of intron 7 of JKB allele which lead to either skipping of exon 6 (JkΔ6 mutation) or exon 7 (JkΔ7 mutation), respectively.^[87,88] In addition, single nucleotide substitutions (C202T, C222A, C582G, T871C, G896A, C956T), 723delA, deletion of intron 3 to intron 5 can also contribute toward a Jk_{null} phenotype.^[86,88-90]

Duffy blood group system

The Duffy antigen chemokine receptor gene is located on the long arm of chromosome 1q22-q23.^[56] There are two alleles; FY**A* and FY**B*, which code for Fya and Fyb antigens, respectively, that are expressed on RBCs and in endothelium, brain, colon, and kidney. They are identified as receptors for *P. vivax* and *P. knowlesi*.^[77,91] The FY**A* and FY**B* alleles differ by a single-point mutation (G125A) that encoding glycine and aspartic acid, respectively.^[92] These alleles determine three of five known phenotypes: Fy (a+b-), Fy (a-b+), and Fy (a+b+).

The fourth phenotype Fy (a-b-) or Fyb^{Es} (erythroid silent) is due to a 67T>C point mutation in the GATA-1-binding motif of the FY**B* promoter.^[91,93] Despite having an apparent Fyb^{Es} phenotype, several individuals do express weak Fyb antigens (Fya-b^{weak}) which are associated with FY**X* allele. This has an Arg89Cys substitution (C286T) in an FY**B* allelic background.^[94]

Dombrock blood group system

The Dombrock antigens Do^a and Do^b are GPs encoded by the DO gene, located on chromosome 12p13.2-p12.1.^[56] These antigens are attached to RBC membrane by glycosylphosphatidylinositol and incompatibility can cause a transfusion reaction.^[95,96] Multiple single nucleotide substitutions in exon 2 of the DO gene: C378T, T624C, and A793G differentiate the Do^a and Do^b alleles.^[97] The first two are silent transitions, but the last one causes an amino acid change at position 265 from asparagine for Do^a to aspartic acid for the Do^b antigen.^[95]

Colton blood group system

The Colton antigens (Co^a and Co^b) are encoded by a gene on chromosome 7p14.^[56,80] The Co^a and Co^b antigens are produced by single nucleotide polymorphism (SNP) (C>T) at nucleotide position 134, resulting in an alanine to valine change.^[98] Alloantibodies against Co^a and Co^b antigens can cause HTR and hemolytic disease of the fetus and newborn.^[96]

Diego blood group system

The Diego antigens (Di^a and Di^b) are versions of the red cell membrane anion exchanger 1 GP and products of *SLC4A1* gene (solute carrier family 4, anion exchanger, member 1) on chromosome 17q12-q21.^[80] A SNP (C2561T) in exon 9 causes an amino acid substitution (Lys658Glu) which differentiates these two antigens.^[56] The Di^a antigen is very rare among Africans and Europeans but more common among Asians and Indigenous Americans.^[96]

Human platelet antigens

The HPAs are GPs found on the surface of anucleate platelets and play major role in hemostasis. Here, platelet aggregation takes place through interaction of HPAs with other vascular endothelium and plasma proteins.^[99] To date, molecular bases of 29 HPAs have been assigned and approved according to guidelines set up by The Platelet Nomenclature Committee.^[100] However, alloantibodies against the high- and low-frequency HPA alleles (designated as a and b, respectively) are only recorded for HPA-1 to -5 and HPA-15 and their molecular bases are given in Table 2.

Human leukocyte antigen

The major histocompatibility complex (MHC) lies on the short arm of human chromosome 6 and encodes a number of immune genes, including HLA Class I and II [Figure 1]. The HLA Class I antigens consist of noncovalently associated of α -chain GP and β_2 -microglobulin.^[101] The α -chain GP determines HLA class specificities (i.e., HLA-A, -C, and -B) and is coded for by the highly polymorphic HLA-A, -C, and -B genes, located within the classical Class I subregion of the MHC complex.^[102] In contrast, the β_2 -microglobulin is nonpolymorphic and encoded by a single gene on chromosome 15.^[103] The HLA Class II antigens are heterodimers of α - and β -chain GPs which are encoded by three pairs of genes on HLA-D region of chromosome 6.^[104] This region codes for the three types of HLA Class II antigens, HLA-DP, -DQ, and -DR.

The HLA Class I antigens are present on all nucleated cells and play roles in endogenous antigenic peptide presentation to CD8⁺ T cells. The peptide-binding clefts of newly synthesized HLA Class I molecules capture endogenously synthesized antigens in the cytosol of the cell. The antigenic peptides associated with HLA Class I molecules are then moved out to the cell surface

Table 2: The molecular bases of human platelet antigen-1 to -5 and -15

Antigen	GP	Chromosome	Nucleotide change	Protein
HPA-1	GPIII ^a	17	176T>C	L33P
HPA-2	GPIb ^a	17	482C>T	T145M
HPA-3	GPII ^b	17	2621T>G	I843S
HPA-4	GPIII ^a	17	506G>A	R143Q
HPA-5	GPI ^a	5	1600A>G	E505K
HPA-15	CD109	6	2108C>A	S703Y

Nucleotide substitutions are shown as changes from a to b alleles. HPA=Human platelet antigen, GP=Glycoprotein

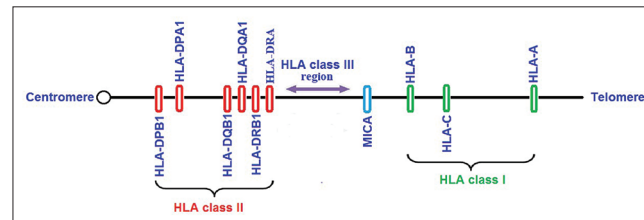


Figure 1: The major histocompatibility complex region on the short arm of human chromosome 6. This region consists of a number of genes and most of them associated with immune responses including human leukocyte antigen Class I and II and major histocompatibility complex Class I chain-related gene A

and form a recognition ligand for T-cell receptors of CD8⁺ T-cytotoxic cell receptors.^[105] In contrast, HLA Class II molecules are usually expressed on the surface of antigen presenting cells and are involved in taking exogenous peptide fragments to CD4⁺ T-cells. The Class II ligands are mainly derived from endocytosed plasma membrane proteins and extracellular fluid proteins. The HLA Class II-peptide complexes are then transported to the cell surface for CD4⁺ T-helper cell recognition.^[106-108] Generally, HLA molecules are involved in recognition of foreign (nonself) peptides, which is crucial for disease defense. However, some foreign materials may contain components that resemble those endogenously expressed by human cells and cause predisposing HLA molecules to bind and present apparent autoantigens to T-cells. This process is known as molecular mimicry and may trigger autoimmune diseases such as ankylosing spondylitis, rheumatoid arthritis, narcolepsy, and coeliac disease.^[109]

Serological and molecular studies have revealed marked differences in levels of variation between α - and β -chains of HLA Class II antigens. For example, the α -chain is constant between individuals for HLA-DR but is polymorphic in HLA-DQ.^[104] Mutation and shuffling of closely linked HLA loci by a recombinational mechanism can account for the extensive diversity of HLA loci.^[110] More than 3390, 4240, 2950, and 1830 of HLA-A, -B, -C, and -DRB1 alleles (respectively) have been reported and compiled in the public HLA database; The IPD and IMGT/HLA database.^[111]

Genotyping of Clinically Relevant Transfusion Markers

It is obvious that the antigens which determine compatibility between donor and recipients are encoded by polymorphic genes^[31,32,45,112] and a wide range of typing platforms are available for consideration, including serology and molecular typing techniques. The first-generation serological testing methods are relatively easy, have short turn-around times, and have become the gold standard in tissue typing laboratories. However, limited supplies of monoclonal antibodies and the broad specificity of serological testing mean that serological technique is not suitable for highly polymorphic antigens. Thus, molecular techniques such as PCR-SSP, PCR-SSO using Luminex, and PCR-SBT are now becoming the preferred means for genetic testing and have their applications been extensively reviewed by others.^[65,113-116] However, the DNA based typing detects genotype, but it is phenotype that is clinically important. Hence, one needs to be absolutely certain that the new methods give results that are confirmed by serology and are cross-checked against each other. Even then, DNA tests (e.g., PCR-SSP, PCR-SSO using Luminex, and SBT) can only detect what they are designed to detect and novel low-frequency variants will inevitably escape (unless one does full genome sequencing). With serology, there is always a chance that the antibodies will pick up novelties including nulls. In other context, the new genomic technology will make it possible to have high resolution genotypic typing for many more people and certainly for all regular donors. As we move toward the era of personal genomics, data will routinely be available for the complete sequences of markers in donors and recipients and we will be able to pick up new variants noting that even SNPs that do not result in amino acid replacements may influence antigen expression levels by changing from a favored to a less favored (i.e., low expression due to scarcity of matching tRNA) codon. Molecular methods show exact variations in particular genes and can improve resolution of polymorphic loci to a precision that could not previously be achieved using serological approaches.^[82,117] In addition, molecular methods are already superior in many ways to the traditional phenotyping and have solved the supply and reliability problems sometimes encountered with antisera and false results produced by poor cell expression and cross-reactivity. We acknowledge that extensive genotyping, including sequencing of multiple blood group loci, may be technically feasible but may not be practical or even necessary for most patients. Nevertheless, it is indisputable that genotyping is a powerful tool to complement and overcome limitations of serology as discussed earlier. In certain scenarios, molecular genotyping can be the only method capable of providing accurate antigen-matched blood units, particularly for multitransfused or highly alloimmunized patients.^[118-122] It is technically complicated to serotype these groups as their peripheral blood contains

donor's transfused red cells,^[123] leading to discrepancies in the test results.^[118,124,125] Furthermore, DNA-based genotyping also facilitates blood typing in patients with the typically weak antigen expression of certain genetic variants,^[126,127] as well as in patients having rare phenotypes where there is difficulty in obtaining or an entire absence of suitable antisera.^[128-130] This form of genotyping has also proved to be superior to conventional serology when it comes to typing of fetal blood types obtained from DNA in maternal plasma.^[131,132] Several molecular platforms that are widely applied for genotyping transplantation and transfusion markers are briefly discussed in the following subsections.

Polymerase chain reaction-Sequence-specific primer

Each reaction mixture for SSP contains PCR master mix, oligonucleotide primer pairs (allele specific and human growth hormone as an internal control), and deoxyribonucleotides. Target amplification is carried out using thermocycler machines (e.g., GeneAmp® PCR System 2700, Applied Biosystems). Agarose gel electrophoresis is then used to detect the presence of allele-specific PCR products of known size and the gel image can be visualized and recorded electronically using an image analyzer (e.g., UVITEC, Cambridge). The presence of specific PCR products reflects the type of allele possessed by an individual. There are many in-house and commercial PCR-SSP kits being developed and tested for blood groups, HPA, and HLA typing; e.g., the studies by Daniels,^[96] Gassner *et al.*,^[133] Gassner *et al.*,^[134] Heymann and Salama,^[135] Prager,^[136] Rozman *et al.*,^[137] and Schaffer and Olerup.^[138]

Polymerase chain reaction-Sequence-specific oligonucleotide using Luminex

In the Luminex-based technique, the target regions are amplified using biotinylated locus-specific primers. Amplicons are then hybridized to complementary bead-bound oligonucleotide probes following denaturation and neutralization procedures. The hybrids are then detected using conjugated streptavidin beads and fluorescence intensities read by flow analyzer. Allele/gene scores are assigned based on reaction patterns of reference standards. This high throughput molecular platform is highly automated and has a rapid turn-around time. It is suitable for large-scale genotyping of alleles encoding for red cell antigens, HPAs, and HLA; e.g., IDCORE^{XT} (Progenika Biopharma, S.A., Spain), IDHPA^{XT} (Progenika Biopharma, S.A.) and LABType® SSO (One Lambda, Inc., CA, USA) for commercially available blood group, HPA, and HLA typing kits, respectively.

Polymerase chain reaction-Sequence-based typing

The PCR-SBT technique is considered as the most accurate molecular technique as it shows the actual sequence of nucleotides in the region of interest. In SBT, the nucleotide

sequences carried by an individual are determined after locus-specific PCR amplification and purification. The purified PCR products are then sequenced by capillary electrophoresis using an automated DNA sequencer such as 3130XL Genetic Analyzer (Life Technologies).

Future Directions of Transfusion Medicine

The future of transfusion medicine is envisaged to be exciting and promising. Modern and advanced technologies are predicted to replace and/or complement the current standard testing regimes. For instance, developments in the field of molecular biology and genetics have already produced great benefits for many branches of clinical practice, including transfusion medicine. The molecular bases of the genes coding for transfusion markers have been elucidated and this has allowed the development applications of various new molecular techniques for donor and recipient matching. This information can now be coupled with the wider availability of cutting-edge tools, such as the next-generation sequencing, and will help give improved insights into the many and varied classes of clinically relevant transfusion genes.^[139-142] This should lead to more accurate phenotypic matching of many transfusion determinants.

It has long been recognized that several genetic markers (e.g., ABO and HLA) determine both, transplantation and transfusion compatibility. Thus, a single and comprehensive registry covering all the significant transplantation and transfusion antigens is expected to become an efficient and important tool for providing safe blood and for searching for a matched organs/stem cells. However, donor recruitment can be challenging especially for multiethnic country.^[116] For any admixed population, it is always important to ensure that all ethnic groups are adequately covered because the frequency of alleles may differ between groups. Further, first-generation admixed people are a special case because they will have markers for two groups, and thus, there will always be a restricted number of suitable donors. Reducing the cost of testing makes it possible to screen larger numbers of people even if they are not making regular donations. One additional issue that may become significant in the future of blood transfusion might be the use of blood substitution products. Synthetic and bioengineered cellular alternatives to natural human blood have shown some initial promise, but it may still be some time before it becomes a routine reality.^[143-145] Until then, blood donations will remain as sacred gifts from altruistic individuals.

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References

1. Learoyd P. The history of blood transfusion prior to the 20th century – Part 1. *Transfus Med* 2012;22:308-14.
2. Pelis K. Moving blood. *Vox Sang* 1997;73:201-6.
3. Allard S. Blood transfusion. *Medicine* 2013;41:242-7.
4. Freedman J. Transfusion – Whence and why. *Transfus Apher Sci* 2014;50:5-9.
5. Refaai MA, Blumberg N. The transfusion dilemma – Weighing the known and newly proposed risks of blood transfusions against the uncertain benefits. *Best Pract Res Clin Anaesthesiol* 2013;27:17-35.
6. World Health Organization. More Voluntary Blood Donation Essentials. Available from: http://www.who.int/mediacentre/news/releases/2012/blood_donation_20120614/en/. [Last accessed on 2016 April 20].
7. National Blood Centre, Ministry of Health Malaysia, editors. *Transfusion Practice Guidelines for Clinical and Laboratory Personnel*. 3rd ed. Ministry of Health Malaysia; 2008.
8. World Health Organization. Countries that Reported Having Achieved 100% Voluntary Non-Remunerated Blood Donation; 2008. Available from: http://www.who.int/bloodsafety/voluntary_donation/countries_100pct_vnrbd_2011.pdf. [Last accessed on 2016 April 20].
9. Cheraghali A. Overview of blood transfusion system of Iran: 2002-2011. *Iran J Public Health* 2012;41:89-93.
10. Jain R, Gupta G. Family/friend donors are not true voluntary donors. *Asian J Transfus Sci* 2012;6:29-31.
11. Domen RE. The ethics of paid versus volunteer blood donation. *J Med Ethics* 1994;20:269-70.
12. World Health Organization. *Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation*. World Health Organization; Geneva. 2012. Available from: <http://www.who.int/iris/handle/10665/76724>. [Last accessed on 2016 April].
13. Mohamed Saleh R. *The Study of the Antibody Response to Malaria Parasites and Its Application to Detect Infected UK Blood Donors*. University of Oxford: Nuffield Clinical Laboratory Science (NDCLS); 2012.
14. Piliavin JA. Temporary deferral and donor return. *Transfusion* 1987;27:199-200.
15. Wevers A, Wigboldus DH, de Kort WL, van Baaren R, Veldhuizen IJ. Characteristics of donors who do or do not return to give blood and barriers to their return. *Blood Transfus* 2014;12 Suppl 1:s37-43.
16. Custer B, Chinn A, Hirschler NV, Busch MP, Murphy EL. The consequences of temporary deferral on future whole blood donation. *Transfusion* 2007;47:1514-23.
17. Greening DW, Glenister KM, Sparrow RL, Simpson RJ. International blood collection and storage: Clinical use of blood products. *J Proteomics* 2010;73:386-95.
18. Green L, Allard S, Cardigan R. Modern banking, collection, compatibility testing and storage of blood and blood components. *Anaesthesia* 2015;70 Suppl 1:3-9, e2.
19. Bolton-Maggs PH, Cohen H. Serious Hazards of Transfusion (SHOT) haemovigilance and progress is improving transfusion safety. *Br J Haematol* 2013;163:303-14.
20. Bihl F, Castelli D, Marincola F, Dodd RY, Brander C.

- Transfusion-transmitted infections. *J Transl Med* 2007;5:25.
21. Niederhauser C, Weingand T, Candotti D, Maier A, Tinguely C, Wuillemin WA, *et al.* Fatal outcome of a hepatitis B virus transfusion-transmitted infection. *Vox Sang* 2010;98:504-7.
 22. Mungai M, Tegtmeier G, Chamberland M, Parise M. Transfusion-transmitted malaria in the United States from 1963 through 1999. *N Engl J Med* 2001;344:1973-8.
 23. World Health Organization. Screening Donated Blood for Transfusion-Transmissible Infections. Switzerland: WHO Press; 1987.
 24. Brecher ME, Hay SN. Bacterial contamination of blood components. *Clin Microbiol Rev* 2005;18:195-204.
 25. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL, *et al.* Bacterial contamination of blood components: Risks, strategies, and regulation: Joint ASH and AABB educational session in transfusion medicine. *Hematology Am Soc Hematol Educ Program* 2003;1:575-89.
 26. Störmer M, Vollmer T. Diagnostic methods for platelet bacteria screening: Current status and developments. *Transfus Med Hemother* 2014;41:19-27.
 27. British Committee for Standards in Haematology, Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, *et al.* Guidelines for pre-transfusion compatibility procedures in transfusion laboratories. *British Committee for Standards in Haematology. Transfus Med* 2013;23:3-35.
 28. Demirkan F, Gunal V, Dereli Y. A new method for electronic crossmatch: ABO/Rh blood group confirmation and antibody screening concomitantly with serologic crossmatch. *Blood Transfus* 2013;122:4833.
 29. Ostendorf N, Niefhoff D, Cassens U, Sibrowski W. Automated serological compatibility testing using a solid-phase test and standard laboratory equipment. *Vox Sang* 2001;80:225-9.
 30. Blann A. *Routine Blood Results Explained*. Cumbria: M & K Update Ltd.; 2006.
 31. Brown CJ, Navarrete CV. Clinical relevance of the HLA system in blood transfusion. *Vox Sang* 2011;101:93-105.
 32. Kekomäki R. Use of HLA- and HPA – Matched platelets in alloimmunized patients. *Vox Sang* 1998;74 Suppl 2:359-63.
 33. Edinur HA, Manaf SM, Che Mat NF. Genetic barriers in transplantation medicine. *World J Transplant* 2016;6:532-41.
 34. Linden JV, Wagner K, Voytovich AE, Sheehan J. Transfusion errors in New York state: An analysis of 10 years' experience. *Transfusion* 2000;40:1207-13.
 35. Myhre BA, McRuer D. Human error—a significant cause of transfusion mortality. *Transfusion* 2000;40:879-85.
 36. Seiden SC, Barach P. Wrong-side/wrong-site, wrong-procedure, and wrong-patient adverse events: Are they preventable? *Arch Surg* 2006;141:931-9.
 37. Alves VM, Martins PR, Soares S, Araújo G, Schmidt LC, Costa SS, *et al.* Alloimmunization screening after transfusion of red blood cells in a prospective study. *Rev Bras Hematol Hemoter* 2012;34:206-11.
 38. Sood R, Makroo RN, Riana V, Rosamma NL. Detection of alloimmunization to ensure safer transfusion practice. *Asian J Transfus Sci* 2013;7:135-9.
 39. Zalpuri S, Zwaginga JJ, van der Bom JG. Risk factors for alloimmunisation after red blood cell transfusions (R-FACT): A case cohort study. *BMJ Open* 2012;2:e001150.
 40. Delaney M, Wendel S, Bercovitz RS, Cid J, Cohn C, Dunbar NM, *et al.* Transfusion reactions: Prevention, diagnosis, and treatment. *Lancet* 2016;388:2825-36.
 41. Strobel E. Hemolytic transfusion reactions. *Transfus Med Hemother* 2008;35:346-53.
 42. Malhotra S, Dhawan HK, Jain A, Sachdev S, Marwaha N. Acute hemolytic transfusion reaction in a patient with bombay phenotype: Implications for ABO grouping. *Indian J Hematol Blood Transfus* 2014;30:108-10.
 43. Park TS, Kim KU, Jeong WJ, Kim HH, Chang CL, Chung JS, *et al.* Acute hemolytic transfusion reactions due to multiple alloantibodies including anti-E, anti-C and anti-JKB. *J Korean Med Sci* 2003;18:894-6.
 44. Metcalfe P. Platelet antigens and antibody detection. *Vox Sang* 2004;87 Suppl 1:82-6.
 45. Rozman P. Platelet antigens. the role of human platelet alloantigens (HPA) in blood transfusion and transplantation. *Transpl Immunol* 2002;10:165-81.
 46. Rafei H, Yunus R, Nassereddine S. Post-transfusion purpura: A case report of an underdiagnosed phenomenon. *Cureus* 2017;9:e1207.
 47. Arewa OP, Nahirniak S, Clarke G. Anti-HPA-1b mediated posttransfusion purpura: A case report. *Case Rep Med* 2013;2013:568364.
 48. Phuangtham R, Romphruk A, Puapairoj C, Leelayuwat C, Romphruk AV. Human platelet antigens in burmese, Karen and North-Eastern Thais. *Transfus Med* 2017;27:60-5.
 49. Roth D. Adverse blood transfusion effects. *J Assoc Vasc Access* 1998;3:10-5.
 50. Dasararaju R, Marques MB. Adverse effects of transfusion. *Cancer Control* 2015;22:16-25.
 51. Giangrande PL. The history of blood transfusion. *Br J Haematol* 2000;110:758-67.
 52. International Society of Blood Transfusion. Available from: <http://www.isbtweb.org>. [Last accessed on 2016 April 25].
 53. Komatsu F, Hasegawa K, Yanagisawa Y, Kawabata T, Kaneko Y, Watanabe S, *et al.* Prevalence of diego blood group dia antigen in mongolians: Comparison with that in Japanese. *Transfus Apher Sci* 2004;30:119-24.
 54. Layrisse M, Arends T. The diego blood factor in chinese and Japanese. *Nature* 1956;177:1083-4.
 55. Dean L. The Kell blood group. In: *Blood Groups and Red Cell Antigens*. Bethesda (MD): National Center for Biotechnology Information (US); 2005.
 56. Reid ME, Lomas-Francis C. *The Blood Group Antigen Facts Book*. 2nd ed. New York: Elsevier Academic Press; 2004.
 57. Langhi DM Jr., Bordin JO. Duffy blood group and malaria. *Hematology* 2006;11:389-98.
 58. Meny GM. The duffy blood group system: A review. *Immunohematology* 2010;26:51-6.
 59. Rowe JA, Opi DH, Williams TN. Blood groups and malaria: Fresh insights into pathogenesis and identification of targets for intervention. *Curr Opin Hematol* 2009;16:480-7.
 60. Zimmerman PA, Ferreira MU, Howes RE, Mercereau-Puijalon O. Red blood cell polymorphism and susceptibility to *Plasmodium vivax*. *Adv Parasitol* 2013;81:27-76.
 61. Saison C, Helias V, Ballif BA, Peyrard T, Puy H, Miyazaki T, *et al.* Null alleles of ABCG2 encoding the breast cancer resistance protein define the new blood group system junior. *Nat Genet* 2012;44:174-7.
 62. Storry Jr., Castilho L, Daniels G, Flegel WA, Garratty G, de Haas M, *et al.* International society of blood transfusion working party on red cell immunogenetics and blood group terminology: Cancun report (2012). *Vox Sang* 2014;107:90-6.
 63. Zelinski T, Coghlan G, Liu XQ, Reid ME. ABCG2 null alleles define the Jr(a-) blood group phenotype. *Nat Genet* 2012;44:131-2.

64. Daniels G. The molecular genetics of blood group polymorphism. *Hum Genet* 2009;126:729-42.
65. Veldhuisen B, van der Schoot CE, de Haas M. Blood group genotyping: From patient to high-throughput donor screening. *Vox Sang* 2009;97:198-206.
66. Harmening DM, Firestone D. The ABO blood group system. In: Harmening DM, editor. *Modern Blood Banking & Transfusion Practices*. 5th ed. Philadelphia: F.A. Davis Company; 2005. p. 108-33.
67. Olsson ML, Chester MA. Polymorphism and recombination events at the ABO locus: A major challenge for genomic ABO blood grouping strategies. *Transfus Med* 2001;11:295-313.
68. Cooling L. ABO, H, and Lewis blood groups and structurally related antigens. In: *Technical Manual*. Vol. 16. Maryland: American Association of Blood Banks, Bethesda; 2008. p. 361-85.
69. Ridgwell K, Spurr NK, Laguda B, MacGeoch C, Avent ND, Tanner MJ, *et al.* Isolation of cDNA clones for a 50 kDa glycoprotein of the human erythrocyte membrane associated with Rh (rhesus) blood-group antigen expression. *Biochem J* 1992;287(Pt 1):223-8.
70. Hemker MB, Ligthart PC, Berger L, van Rhenen DJ, van der Schoot CE, Wijk PA, *et al.* DAR, a new RHD variant involving Exons 4, 5, and 7, often in linkage with ceAR, a new Rhce variant frequently found in African blacks. *Blood* 1999;94:4337-42.
71. Arce MA, Thompson ES, Wagner S, Coyne KE, Ferdman BA, Lublin DM, *et al.* Molecular cloning of rhD cDNA derived from a gene present in rhD-positive, but not rhD-negative individuals. *Blood* 1993;82:651-5.
72. Daniels G. The molecular genetics of blood group polymorphism. *Transpl Immunol* 2005;14:143-53.
73. Wiler M. The Rh blood group system. In: Harmening DM, editor. *Modern Blood Banking & Transfusion Practices*. 5th ed. Philadelphia: F.A. Davis Company; 2005. p. 134-147.
74. Grunbaum BW, Selvin S, Myhre BA, Pace N. Distribution of gene frequencies and discrimination probabilities for 22 human blood genetic systems in four racial groups. *J Forensic Sci* 1980;25:428-44.
75. Huang CH, Reid M, Daniels G, Blumenfeld OO. Alteration of splice site selection by an exon mutation in the human glycoprotein A gene. *J Biol Chem* 1993;268:25902-8.
76. Velliquette RW, Hu Z, Lomas-Francis C, Hue-Roye K, Allen JL, Mirabella D, *et al.* Novel single-nucleotide change in GYP*A in a person who made an alloantibody to a new high-prevalence MNS antigen called ENEV. *Transfusion* 2010;50:856-60.
77. Leger RM, Calhoun L. Other major blood group systems. In: Harmening DM, editor. *Modern Blood Banking & Transfusion Practices*. 5th ed. Philadelphia: F.A. Davis Company; 2005.
78. Poole J, Banks J, Bruce LJ, Ring SM, Levene C, Stern H, *et al.* Glycophorin A mutation ala65 -> pro gives rise to a novel pair of MNS alleles ENEP (MNS39) and HAG (MNS41) and altered Wrb expression: Direct evidence for GPA/band 3 interaction necessary for normal wrb expression. *Transfus Med* 1999;9:167-74.
79. Lee S, Russo DC, Reiner AP, Lee JH, Sy MY, Telen MJ, *et al.* Molecular defects underlying the kell null phenotype. *J Biol Chem* 2001;276:27281-9.
80. Daniels G. *Human Blood Groups*. 2nd ed. Malden, MA: Blackwell Science; 2002.
81. Lee S, Russo DC, Reid ME, Redman CM. Mutations that diminish expression of kell surface protein and lead to the kmod RBC phenotype. *Transfusion* 2003;43:1121-5.
82. Patnaik SK, Helmberg W, Blumenfeld OO. BGMUT: NCBI dbRBC database of allelic variations of genes encoding antigens of blood group systems. *Nucleic Acids Res* 2012;40:D1023-9.
83. Lee S, Wu X, Reid M, Zelinski T, Redman C. Molecular basis of the kell (K1) phenotype. *Blood* 1995;85:912-6.
84. Lee S. The value of DNA analysis for antigens of the kell and KX blood group systems. *Transfusion* 2007;47:32S-9S.
85. Olivès B, Merriman M, Bailly P, Bain S, Barnett A, Todd J, *et al.* The molecular basis of the kidd blood group polymorphism and its lack of association with type 1 diabetes susceptibility. *Hum Mol Genet* 1997;6:1017-20.
86. Irshaid NM, Henry SM, Olsson ML. Genomic characterization of the kidd blood group gene: Different molecular basis of the JK(a-b-) phenotype in polynesians and finns. *Transfusion* 2000;40:69-74.
87. Lin M, Yu LC. Frequencies of the JKnull (IVS5-1g>a) allele in Taiwanese, Fujian, Filipino, and Indonesian populations. *Transfusion* 2008;48:1768.
88. Lucien N, Sidoux-Walter F, Olivès B, Moulds J, Le Pennec PY, Cartron JP, *et al.* Characterization of the gene encoding the human kidd blood group/urea transporter protein. evidence for splice site mutations in JKnull individuals. *J Biol Chem* 1998;273:12973-80.
89. Liu HM, Lin JS, Chen PS, Lyou JY, Chen YJ, Tzeng CH, *et al.* Two novel jk(null) alleles derived from 222C>A in exon 5 and 896G>A in exon 9 of the JK gene. *Transfusion* 2009;49:259-64.
90. Wester ES, Johnson ST, Copeland T, Malde R, Lee E, Storry JR, *et al.* Erythroid urea transporter deficiency due to novel JKnull alleles. *Transfusion* 2008;48:365-72.
91. Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in duffy-negative individuals. *Nat Genet* 1995;10:224-8.
92. Estalote AC, Proto-Siqueira R, Silva WA Jr., Zago MA, Palatnik M. The mutation G298A--&Ala100Thr on the coding sequence of the Duffy antigen/chemokine receptor gene in non-caucasian Brazilians. *Genet Mol Res* 2005;4:166-73.
93. Rios M, Chaudhuri A, Mallinson G, Sausais L, Gomensoro-Garcia AE, Hannon J, *et al.* New genotypes in Fy(a-b-) individuals: Nonsense mutations (Trp to stop) in the coding sequence of either FY A or FY B. *Br J Haematol* 2000;108:448-54.
94. Tournamille C, Le Van Kim C, Gane P, Le Pennec PY, Roubinet F, Babinet J, *et al.* Arg89Cys substitution results in very low membrane expression of the duffy antigen/receptor for chemokines in Fy(x) individuals. *Blood* 1998;92:2147-56.
95. Costa FP, Hue-Roye K, Sausais L, Velliquette RW, Da Costa Ferreira E, Lomas-Francis C, *et al.* Absence of DMR, a new antigen in the dombrock blood group system that weakens expression of Do(b), Gy(a), Hy, Jo(a), and DOYA antigens. *Transfusion* 2010;50:2026-31.
96. Daniels G. Other blood groups. In: Roback JD, Combs MR, Grossman BJ, Hillyer CD, editors. *Technical Manual*. 16th ed. Maryland: American Association of Blood Banks, Bethesda; 2008. p. 411-98.
97. Storry Jr., Westhoff CM, Charles-Pierre D, Rios M, Hue-Roye K, Vege S, *et al.* DNA analysis for donor screening of dombrock blood group antigens. *Immunohematology* 2003;19:73-6.
98. Arnaud L, Helias V, Menanteau C, Peyrard T, Lucien N, Ripoche P, *et al.* A functional AQP1 allele producing a Co(a-b-) phenotype revises and extends the colton blood group system. *Transfusion* 2010;50:2106-16.

99. Norton A, Allen DL, Murphy MF. Review: Platelet alloantigens and antibodies and their clinical significance. *Immunohematology* 2004;20:89-102.
100. Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, *et al.* Nomenclature of human platelet antigens. *Vox Sang* 2003;85:240-5.
101. Eren E, Travers P. The structure of the major histocompatibility complex and its molecular interactions. In: Rand L, Warrens A, editors. *HLA in Health and Disease*. 2nd ed. San Francisco, New York: Academic Press; 2000. p. 3-33.
102. Milner CM, Campbell RD, Trowsdale J. Molecular genetics of the human major histocompatibility complex. In: Rand L, Warrens A, editors. *HLA in Health and Disease*. 2nd ed. San Francisco, New York: Academic Press; 2000. p. 35-50.
103. Goodfellow PN, Jones EA, Van Heyningen V, Solomon E, Bobrow M, Miggiano V, *et al.* The beta2-microglobulin gene is on chromosome 15 and not in the HL-A region. *Nature* 1975;254:267-9.
104. Trowsdale J. Genetics and polymorphism: Class II antigens. *Br Med Bull* 1987;43:15-36.
105. Thorsby E. MHC structure and function. *Transplant Proc* 1999;31:713-6.
106. Crumpton MJ. Introduction. *Br Med Bull* 1987;43:1.
107. Davies H. *Introductory Immunobiology*. London: Chapman & Hall; 1997.
108. Strominger JL. Structure of class I and class II HLA antigens. *Br Med Bull* 1987;43:81-93.
109. Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: The failure of self tolerance. *Science* 1990;248:1380-8.
110. Bodmer W, Thomson G. Population genetics and evolution of the HLA system. In: Jand D, Svejgaard A, editors. *HLA and Disease*. Copenhagen: Munksgaard; 1977. p. 280-95.
111. Robinson J, Halliwell JA, Hayhurst JD, Flicke P, Parham P, Marsh SG, *et al.* The IPD and IMGT/HLA database: Allele variant databases. *Nucleic Acids Res* 2015;43:D423-31.
112. Engelfriet CP, Reesink HW, Aster RH, Brand A, Tomson B, Claas FH, *et al.* Management of alloimmunized, refractory patients in need of platelet transfusions. *Vox Sang* 1997;73:191-8.
113. Curtis BR, McFarland JG. Detection and identification of platelet antibodies and antigens in the clinical laboratory. *Immunohematology* 2009;25:125-35.
114. Veldhuisen B, Porcelijn L, Ellen van der Schoot C, de Haas M. Molecular typing of human platelet and neutrophil antigens (HPA and HNA). *Transfus Apher Sci* 2014;50:189-99.
115. Edinur HA, Chambers GK, Dunn PP. Recent developments in transplantation and transfusion medicine. *Ann Transplant* 2015;20:424-9.
116. Dunn PP. Human leucocyte antigen typing: Techniques and technology, a critical appraisal. *Int J Immunogenet* 2011;38:463-73.
117. Robinson J, Halliwell JA, McWilliam H, Lopez R, Parham P, Marsh SG, *et al.* The IMGT/HLA database. *Nucleic Acids Res* 2013;41:D1222-7.
118. Bakanay SM, Ozturk A, Ileri T, Ince E, Yavasoglu S, Akar N, *et al.* Blood group genotyping in multi-transfused patients. *Transfus Apher Sci* 2013;48:257-61.
119. da Costa DC, Pellegrino J Jr., Guelsin GA, Ribeiro KA, Gilli SC, Castilho L, *et al.* Molecular matching of red blood cells is superior to serological matching in sickle cell disease patients. *Rev Bras Hematol Hemoter* 2013;35:35-8.
120. Denomme GA. Prospects for the provision of genotyped blood for transfusion. *Br J Haematol* 2013;163:3-9.
121. Wagner FF. Why do we use serological blood group phenotype determination in chronically transfused patients? *Blood Transfus* 2014;12:1-2.
122. Belsito A, Costa D, Fiorito C, De Iorio G, Casamassimi A, Perrotta S, *et al.* Erythrocyte genotyping for transfusion-dependent patients at the azienda universitaria policlinico of naples. *Transfus Apher Sci* 2015;52:72-7.
123. Avent ND. Large-scale blood group genotyping: Clinical implications. *Br J Haematol* 2009;144:3-13.
124. Castilho L, Rios M, Bianco C, Pellegrino J Jr., Alberto FL, Saad ST, *et al.* DNA-based typing of blood groups for the management of multiply-transfused sickle cell disease patients. *Transfusion* 2002;42:232-8.
125. Castilho L, Rios M, Pellegrino J Jr., Saad S, Costa F. Blood group genotyping facilitates transfusion of beta-thalassemia patients. *J Clin Lab Anal* 2002;16:216-20.
126. McGowan EC, Lopez GH, Knauth CM, Liew YW, Condon JA, Ramadi L, *et al.* Diverse and novel RHD variants in Australian blood donors with a weak D phenotype: Implication for transfusion management. *Vox Sang* 2017;112:279-87.
127. Moraes-Souza H, Alves VM. Impact on patient of the detection of weakly expressed RHD antigens in blood donors. *Rev Bras Hematol Hemoter* 2015;37:290-1.
128. Baumgarten R, van Gelder W, van Wintershoven J, Maaskant-Van Wijk PA, Beckers EA. Recurrent acute hemolytic transfusion reactions by antibodies against D_oa antigens, not detected by cross-matching. *Transfusion* 2006;46:244-9.
129. Chen Q, Li J, Xiao J, Du L, Li M, Yao G, *et al.* Molecular genetic analysis and structure model of a rare B(A)₀2 subgroup of the ABO blood group system. *Transfus Apher Sci* 2014;51:203-8.
130. Lopez GH, Mcbean RS, Wilson B, Irwin DL, Liew YW, Hyland CA, *et al.* Molecular typing for the Indian blood group associated 252G>C single nucleotide polymorphism in a selected cohort of Australian blood donors. *Blood Transfus* 2015;13:78-85.
131. Boggione CT, Luján Brajovich ME, Mattaloni SM, Di Mónaco RA, García Borrás SE, Biondi CS, *et al.* Genotyping approach for non-invasive foetal RHD detection in an admixed population. *Blood Transfus* 2016;15:66-73.
132. Papasavva T, Martin P, Legler TJ, Liasides M, Anastasiou G, Christofides A, *et al.* Prevalence of RHD status and clinical application of non-invasive prenatal determination of fetal RHD in maternal plasma: A 5 year experience in Cyprus. *BMC Res Notes* 2016;9:198.
133. Gassner C, Schmarda A, Kilga-Nogler S, Jenny-Feldkircher B, Rainer E, Müller TH, *et al.* RHD/CE typing by polymerase chain reaction using sequence-specific primers. *Transfusion* 1997;37:1020-6.
134. Gassner C, Schmarda A, Nussbaumer W, Schönitzer D. ABO glycosyltransferase genotyping by polymerase chain reaction using sequence-specific primers. *Blood* 1996;88:1852-6.
135. Heymann GA, Salama A. Sequence-specific primers for MNS blood group genotyping. *Blood Transfus* 2010;8:159-62.
136. Prager M. Molecular genetic blood group typing by the use of PCR-SSP technique. *Transfusion* 2007;47:54S-9S.
137. Rozman P, Dovc T, Gassner C. Differentiation of autologous ABO, RHD, RHCE, KEL, JK, and FY blood group genotypes by analysis of peripheral blood samples of patients who have recently received multiple transfusions. *Transfusion* 2000;40:936-42.
138. Schaffer M, Olerup O. HLA-AB typing by polymerase-chain reaction with sequence-specific primers: More accurate, less errors, and increased resolution compared to serological typing. *Tissue Antigens* 2001;58:299-307.

139. Liu Z, Liu M, Mercado T, Illoh O, Davey R. Extended blood group molecular typing and next-generation sequencing. *Transfus Med Rev* 2014;28:177-86.
140. Tilley L, Grimsley S. Is next generation sequencing the future of blood group testing? *Transfus Apher Sci* 2014;50:183-8.
141. Johnsen JM. Using red blood cell genomics in transfusion medicine. *Hematology Am Soc Hematol Educ Program* 2015;2015:168-76.
142. Fichou Y, Audrézet MP, Guéguen P, Le Maréchal C, Férec C. Next-generation sequencing is a credible strategy for blood group genotyping. *Br J Haematol* 2014;167:554-62.
143. Seifried E, Mueller MM. The present and future of transfusion medicine. *Blood Transfus* 2011;9:371-6.
144. Chen JY, Scerbo M, Kramer G. A review of blood substitutes: Examining the history, clinical trial results, and ethics of hemoglobin-based oxygen carriers. *Clinics (Sao Paulo)* 2009;64:803-13.
145. Scott MG, Kucik DF, Goodnough LT, Monk TG. Blood substitutes: Evolution and future applications. *Clin Chem* 1997;43:1724-31.

