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Review

# Emerging strategies of blood group genotyping for patients with hemoglobinopathies

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ABSTRACT

Red cell alloimmunization is a serious problem in chronically transfused patients. A number of high-throughput DNA assays have been developed to extend or replace traditional serologic antigen typing. DNA-based typing methods may be easily automated and multiplexed, and provide reliable information on a patient. Molecular genotyping promises to become cheaper, being not dependent on serologic immunoglobulin reagents. Patients with hemoglobinopathies could benefit from receiving extended genomic typing. This could limit post transfusional complications depending on subtle antigenic differences between donors and patients. Patient/donor compatibility extended beyond the phenotype Rh/Kell may allows improved survival of transfused units of red blood cells (RBC) and lead to reduced need for blood transfusion and leading to less iron overload and reduced risk of alloimmunization. Here we discuss the advantages and limitations of current techniques, that detect only predefined genetic variants. In contrast, target enrichment next-generation sequencing (NGS) has been used to detect both known and de novo genetic polymorphisms, including single-nucleotide polymorphisms, indels (insertions/deletions), and structural variations. NGS approaches can be used to develop an extended blood group genotyping assay system.



**Contents**



## 1. Introduction

In patients receiving repeated blood transfusions, alloimmunization and iron overload may be major clinical complications that could limit further therapies. The production of alloantibodies and/or autoantibodies, complicates RBC cross-matching, shortens RBC survival, delays provision of safe transfusions and may

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accelerate tissue iron loading. DNA-based typing methods are considered promising tools to improve transfusion therapy, decreasing antibody formation to blood group antigens whenever a typing discrepancy between donor and recipient is present.

DNA sequence variations at 45 loci produces 346 serologically distinct RBC antigen phenotypes recognized by the International Society of Blood Transfusion (ISBT) [1] (Table 1). Polymorphic RBC antigens may be proteins, carbohydrates or lipids exposed on cell membrane. The transfusion of a variant protein epitope recognized as "non-self" may be tolerated in some individuals or generates adverse immunological reactions in others. To predict and control these events, DNA assays have been increasingly implemented in immunohematology [2,3], also due to an exponential growth of available genomic information. DNA-based typing methods (genotyping) may be used in addition to traditional serological method (phenotyping). DNA-based genotyping allows the accurate prediction of multiple erythrocyte antigens by evaluating the presence of known single nucleotide polymorphisms (SNPs) and other variations. The serological methods presents same limitations especially in chronically transfused patients. For exampe, the presence of circulating transfused RBCs with a positive direct antiglobulin test (DAT) and alloantibodies. Moreover, serological methods fail to identify accurately suspected variants, weak expression or null phenotype of antigens or discrepancies in blood group determination. DNA-based methods allow extensive and unbiased analysis and the identification of antigens and allelic variants.

Until now, SNPs with clinical significance beyond those of the ABO and Rh systems, have been manually tested by PCR with specific oligonucleotides or through the use of restriction enzymes or robotically by microarrays. These methods although robust, are being replaced by higher-throughput Next-Generation Sequencing (NGS) applications. The use of the new technologies will allow the routine genotyping ofdonors and patients for all the clinically relevant polymorphisms, with important advantages in the reduction of the genetic component of alloimmunization [4–6]. This information may be used to reduce the incidence of acute and delayed hemolytic reactions (HTR) The spread of NGS technologies have the ability to sequence DNA at unprecedented speed and detail, it may turn out to be a challenge to have enough blood units with correct extended genotype for all chronically transfused patients.

## 2. Genetic variants associated with blood group antigen

The blood group antigens are the product of alleles of a single locus or very close loci: each allelic structure is transmitted to the children as clusters. Erythrocyte antigens and their blood groups belong to different structures of membrane, glycoproteins or glycolipids in the case of ABO, P, Lewis, H, I, and Globoside system, their corresponding genes encoding glycosyltransferases, which catalyze the synthesis and transfer of specific monosaccharides. A and B transferase are encoded by the ABO gene localized on chromosome 9 and organized in seven exons and a single nucleotide change in an A or B allele can result in an inactive transferase and a group O phenotype. Genotyping methods have been developed to decrease the risk for erroneous ABO prediction [7–12]. Genetic diversity of the Rh locus has been revealed in the last decade, to assess the presence of molecular changes that can cause more than 200 *RHD* and 80 *RHCE* alleles reported [13–15]. Particularly, the *RHD* genotyping provides the opportunity to assess the presence of molecular changes can cause weak or silenced (null) D due to alternative splicing, premature stop codons hybrid genes or promoter silencing. Partial D phenotypes are characterized by loss of epitopes and people with partial D may form anti-D when exposed to regular D antigen. Within D-variants, DAR, DIIIa, DIVa and some DAU types can lead to anti-D alloimmunization in African patients with Sickle cell disease (SCD). Variant RH alleles may encode the low incidence antigens such as the V, VS antigen expressed in about 26–40% of African and the $Js^a$ and the $Co^b$ antigens that are generally not routinely typed but are potentially immunogenic [16]. While individuals with altered *RHD* encoding weak D, defined as expressing a reduced amount of D antigen but not lacking epitopes, are not typically at risk for D sensitization. Some variants are associated with the *RHCE* gene such as partial "C" and partial "e" antigens. Systematic analysis of molecular *RHD* and *RHCE* performed in blood donors and patients is important for improving transfusion therapy because the D antigen is the most immunogenic blood group antigen. Anti-D antibody is still the antibody most commonly implicated in hemolytic disease of the fetus and newborn. Rh-D variants are often difficult to define serologically without DNA-based Rh typing; which could be very helpful to improve the match between donor and recipient especially for transfusion-dependent patients with hemoglobinopathies [17–19]. The Kell antigen is the product of a single gene, *KEL*, spanning 19 exons on chromosome 7, and consist of 32 antigens; K differs from k (KEL2, Cellano) by a single nucleotide change in exon 6, resulting in threonine in k and methionine in K at position 193.

Kell antigen is important in transfusion medicine after ABO and Rh, the immunogenicity of the K antigen is second only to the ABO and D antigen, and anti-K is usually IgG, and potentially clinically significant, causing transfusion reactions and possible hemolytic disease of the fetus and newborn (HDFN) [20–23]. Antibody titres do not correlate with severity in HDFN due to anti-K in contrast to HDFN due to anti-D. The clinical picture of HDFN due to anti-K differs somewhat from that due to anti-D, with cases usually (but not always) being less severe [24]. The Duffy (Fy) blood group antigens are the product of a gene on chromosome 1q23.2 that comprises two coding exons [25,26]. The system is defined by two codominant alleles: FY1 and FY2 that are polymorphic in the population and encode two antithetical antigens, Fy(a) and Fy(b). The Duffy-negative phenotype Fy (a-b-), is prevalent in African and American black population. The Duffy silencing mutation in the region (-67T > C) of GATA box at the promoter region prevents the expression of Duffy glycoprotein in the erythrocytes while preserving the expression of Fy(a) and/or Fy(b) in other tissues [27]. The c.265C > T on the FY*B allele causes the expression of allele Fy(bw). In several cases Fy(a + b-) was found when examined with the serological method, while Fy(a + bw) was revealed by the molecular assay. Because of the immunogenicity of Duffy gene, there could be important clinical effects in transfusion of a Fy(bw) blood unit to a multi-transfused patient with antibodies anti-Fy(b) but so far there are no reported clinical consequences [28]. The MNS (M/N, S/s) blood group system as the Rh blood group system, is highly complex with 48 antigens currently recognized by the ISBT. The most important antigens of this system are: M, N, S, s and U. These are products of three highly homologous genes glycophorin A (GYPA), B (GYPB) and E (GYPE), localized on chromosome 4 (4q31.21). Antigen-carrying glycophorins are major red blood cell membrane sialoglycoproteins and their antigenic manifestation involves both protein sequences and attached glycans [29,30]. The U-negative individual is not routinely transfused with U-negative blood and Anti-U identifies a high-frequency antigen and causes immediate and delayed HTRs. $Mi^a$ and Mur antigens (Miltenberger antigens) resulting from recombination of the glycophorin A and B genes (hybrid glycophorins), anti-$Mi^a$/Mur is most prevalent in East Asians, e.g. Taiwanese 7.3%, Hong Kong Chinese 6·3% and Thai 8% [31–34]. The Kidd blood group system is the product of the *SLC14A1* (JK) gene localized on chromosome 18q11-q12. The gene comprises 11 exons distributed over 30 kbp of gDNA and code for an Urea transporter (HUT11) [35,36]. The rare Jk(a-b-) null phenotype can be caused by homozygosity for a silent JK allele and anti-Jk3 can be found after immunization causing acute and