Applying molecular immunohematology discoveries to daily transfusion practice

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The discovery of the molecular basis of most red cell antigens, combined with advances in molecular testing, have paved the way for the use of genotyping to predict the red cell phenotype. Genotyping offers many advantages over serologic testing of recipients' blood with the primary benefit being able to predict the blood group phenotype in situations that do not permit this serologically.

Alloantibodies develop in approximately 2-4 percent of people after transfusion, with a higher rate (20-40 percent) among chronically transfused patients such as those with sickle cell anemia or thalassemia. Using hemagglutination to phenotype red blood cells from a person with alloantibodies can be complex and time consuming and the results can be difficult to interpret. In these cases, genotyping helps to predict the red cell antigen type and provides a more complete characterization of the blood type.

The clinical benefits for the transfusion-dependent population are tangible: better matched blood decreases the risk of hemolytic transfusion reactions, especially delayed transfusion reactions to existing alloantibodies, and prevents new instances of alloimmunization. Additionally, the use of better matched blood can reduce transfusion requirements, decreasing the risk of transfusion-related acute lung injury and potential exposure to infectious diseases.

According to our experience, matching at DNA level can provide an added level of safety and efficacy for chronically transfused patients. Genotyping can also help to decrease costs, in both terms of time and money, associated with complex serologic workups and a higher number of transfusions.

In summary, molecular testing is a rapidly advancing field that offers tremendous potential in transfusion medicine and is proving to be a powerful tool to help serology, with the potential advantages of identifying rare blood and finding antigen matches for chronically transfused patients.

However, it is important to remember that, regardless of the test protocols used, genotyping can only predict a blood type. In rare situations a genotype determination will not correlate with the antigen expression on the red blood cell and appropriate assays are necessary to detect nucleotide changes that alter the predicted phenotype. Given the large number of known genetic events that silence or weaken the expression of antigens encoded by an allele, it will be a long time before all relevant nucleotide changes are revealed for all blood group systems in all ethnic groups. For these reasons, care must be taken when using molecular methods in antibody investigations as the serological problem may involve the inheritance of a null allele, a hybrid gene or a new variant.

In this context, the analysis of blood group variants in a specific population is very important to predict a red cell phenotype using molecular methods. The more we learn about frequency and molecular background of such variants in different populations, the more accurate the genotype/phenotype results will be. For example, more work needs to be done to show that the molecular method used can recognize variants seen primarily in the African population. Two common examples are the GATA-promoter polymorphism, which silences the *FY*B* gene and two different *GYPB* alleles (*GYPB*P2* and *GYPB*NY*) that silence the expression of the S antigen.

The S-s- phenotype, with rare exception, is found only in persons of African descent and may be associated with the absence or weak expression of the high U antigen frequency. S-s- persons can develop anti-U, which is known to cause decreased survival of transfused antigen-positive red blood cells. Therefore, it is very important to predict those phenotypes correctly. We have already shown in a specific blood donor population of African Brazilians that the S- and S-s-U^{var} phenotypes can be associated with *GYPB*P2* (73%) and *GYPB*NY* (27%) alleles (variant forms of *GYPB*)[1]. In this issue, there is a paper on an admixed population of Minas Gerais showing that the S- and S-s-U^{var} phenotypes are associated with the *GYPB*P2* allele characterized by a g>t mutation at +5 of the intron 5 donor splice site[2]. Therefore, assays for routine analysis of *GYPB* in this population must include this single nucleotide polymorphism (SNP) in the final prediction of the phenotype in order to avoid false positive

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results. This is a very interesting discovery that will help to apply blood group genotyping to standard practices. The available wealth of serologically defined variants has contributed to the rapid rate at which the genetic diversity of blood group genes has been revealed.

The pattern of genetic diversity of a specific genomic region depends both on the demographic history of populations and on locus specific evolutionary factors such as mutations, recombination and natural selection[3]. The pattern of nucleotide diversity of *GYPB* is also informative about the role of S/s alleles in susceptibility to malaria infection, because it allows inferences about the action of natural selection driven by malaria during human evolution[4].

In addition, molecular immunohematology is providing us with the opportunity to implement a novel set of tools to personalize medicine and to adapt blood products to the clinical needs of patients.

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