# Comments on: Molecular matching of red blood cells is superior to serological matching in sickle cell disease patients

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An important study by da Costa et al.[1] regarding the use of blood group antigen genotyping to obtain improved matching of blood donors for sickle cell disease (SCD) patients is presented in this issue of the Revista Brasileira de Hematologia e Hemoterapia. There are very few available prophylactic treatment options for SCD morbidity which includes painful vaso-occlusive crises and stroke. Hydroxyurea therapy, which is not effective in all patients, and either simple or exchange transfusion, are the most common preventative modalities. The objective of chronic transfusion is to dilute hemoglobin (Hb) S with Hb A and achieve a prolonged elevation of hemoglobin and hematocrit levels. Exchange transfusion may have the added effect of removing soluble inflammatory and coagulation factors. Besides the risk of iron overload associated with chronic transfusion, patients are at risk of alloimmunization that can cause delayed hemolytic transfusion reactions and hyperhemolysis syndrome. A significant fraction of SCD patients treated with transfusions have antibodies to red cell antigens. A patient's antibody profile can make donor unit selection difficult, potentially resulting in delays and increased cost of multiple antigen-negative red cell units.

As the authors explain, there is no standard of care for matching donors to chronically-transfused patients with SCD. In the United States, the commonality of antibodies to C, E and K antigens in this heavily African-American patient population coupled with the prevalence of these antigens in the Caucasian population and rarity in the African-American population lead to the establishment of programs that match C, E and K antigens in these patients. The American Red Cross and the Children's Hospital of Philadelphia launched a program in 1996 in which voluntary African-American blood donors can select a tie tag to be attached to their unit such that it can be directed for the treatment of a child with SCD[2]. Similar programs were established at other centers[3-5]. A recent series of articles reviewed the state of SCD transfusion programs at several major treatment centers in the United States[6-13].

The genetic basis for many blood group antigens is known[14]. Many antigens are due to single nucleotide polymorphisms (SNPs). This has allowed for the generation of multianalyte panels that can interrogate multiple SNPs simultaneously to predict blood group antigens[15]. In the past several years, the use of genotyping to predict blood group antigens has become more common in blood centers and in some large hospitals. When and how this information is used in the care of patients with SCD varies. For example, some sickle cell protocols obtain a basic red cell phenotype prior to the start of chronic transfusion and match for C, E and K antigens, and extend the match after the patient demonstrates antibodies to other antigens. Since antibodies to the Duffy, Kidd and MNS antigens are common in alloimmunized SCD patients, there has been discussion of extending the match to these antigens.

Klapper et al. used the Human Erythrocyte Antigen (HEA) BeadChip™ multianalyte genotyping platform paired with a web-based inventory management system to model a hospital blood bank inventory with the population of SCD patients[16]. This virtual study found that matching for the major antigens in the RH, KEL, FY, JK and MNS systems would be possible at least 50% of the time.

The authors da Costa et al. take this type of modeling and operationalize it[1]. They explored the use of molecular genotyping in matching their blood donors and 35 SCD patients. They selected one hundred and ten donor units serologically matched for ABO, Ca, Ee and K antigen status as well as based on the presence of antibodies. Next, they examined the exactness of the match by exploring the genotype information available for these donors. In addition to C, c, E, e, K, $Fy^a$, $Fy^b$, $Jk^a$, $Jk^b$, S, s and $Di^a$ predicted phenotypes generated by the HEA BeadChip™, they performed additional assays [polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequence analysis] to characterize *RH* variants. The authors used a custom web-hosted inventory management system that identifies the most compatible match.

The results of their study are very informative in several regards. Of the 35 patients, 21 had discrepancies or mismatches for multiple antigens when compared to the blood units matched for them serologically with the majority of discrepancies or mismatches in the RH, FY, JK and

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MNS systems. Thus, with the use of the serologically-matched blood, these patients would be at risk of alloimmunization to these antigens even though alternative blood was available that extended the match to these antigens.

Next, the authors report that eight Rh alloimmunized patients had *RHD* and *RHCE* variants that cannot be identified serologically. Importantly, these variants cannot be identified by the HEA BeadChip™ method and in this study were identified by PCR-RFLP and DNA sequence analysis. There are more than 300 *RH* variants and genotyping and allele assignments can be challenging. However, in this study, the authors suggest that their genotyped donors include *RH* variants that were used to match these patients.

Overall, the authors report that their genotype-matching program resulted in elevated hemoglobin levels, increased time between transfusions and a lack of newly developed alloantibodies. They point out that though it is not feasible to provide a complete antigen-match blood transfusion to all SCD patients for all antigens in their institution, matching for C, c, E, e, K, S, Fya, $Jk^a$ and $Jk^b$ antigens is cost-effective for the treatment of chronically transfused SCD patients.

At the American Red Cross, we have been screening large numbers of African-American blood donors to supply antigen-negative blood to SCD patients. An outcome of such screening has been the identification of discrepancies in the serologic phenotype and the genotype-predicted phenotype. In such cases it is important to determine which type is correct for the purposes of transfusion. If such discrepancies cannot be resolved, donors should be considered positive for the antigen in question while patients should be considered negative. Furthermore, if a discrepancy is discovered that impacts a donor phenotype, it is imperative to review the recipients of past donations and their transfusion outcomes including the antibodies identified.

The American Rare Donor Program, a consortium of American Red Cross and AABB-accredited immunohematology reference laboratories that aid in matching donor units to patients with antibodies, has been using molecular genotype information for several years[17]. Most recently, they have begun requiring molecular genotype information for donors reported to be "$hr^B$-negative". This is important as there are many *RHCE* variants that are $hr^B$-negative[18] and there is no SNP that by itself identifies an individual as $hr^B$-negative. Further, the importance of *RH* characterization of SCD patients is supported by the frequency of antibodies to *RH* antigens for which the patient appears positive, yet molecular characterization identifies a variant. While the study by da Costa et al.[1] is small, it illustrates the feasibility of matching patients based on *RH* variants as well as based on extending the antigen match to include FY, JK and MNS.

The use of molecular testing to predict blood group antigens extends beyond patients with SCD to other multiply-transfused patient populations. Also, it is a useful tool for phenotype prediction in patients with warm autoantibodies or a positive direct antiglobulin test who are routinely antigen tested using the anti-human globulin (AHG) technique. Further, it is useful in patients with autoimmune hemolytic anemia.

We agree with most of the conclusions drawn by the authors and look forward to more and larger studies such as this one that demonstrate the outcomes of extended matching programs. Specifically, data that show the effect of such programs on hemoglobin level, transfusion frequency and alloimmunization rates. As there is a cost associated with the establishment of a donor population with readily-available genotype information as well as genotyping the patient, data is important in discussions of the return on investment associated with genotyping. Finally, the computer-based tool that was used to identify the most compatible unit is something that will become increasingly necessary as genotype matching programs grow and as the importance of matching for *RH* variants is appreciated.

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