Noninvasive Buccal Swab Antigen Sample and Molecular Testing Provides Extended Antigen Typing for Patients with Hemoglobinopathies

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Objective To demonstrate the feasibility of performing a noninvasive, molecular-based red blood cell (RBC) antigen test on infants and very young children with sickle cell disease as part of a statewide newborn screening follow-up program.

Study design A prospective pilot project was conducted using a noninvasive buccal swab and test kit to perform DNA-based, extended RBC phenotyping in 92 children participating in a newborn hemoglobinopathy screening follow-up program. Reported data include the extended panel of antigens detected by molecular analysis compared with unaffected population estimates.

Results Molecular-based RBC antigen testing was successful, with extended RBC typing generated for all subjects. Molecular testing detected several rare negative or rare positive phenotypes, demonstrating the utility of obtaining an extended antigen panel.

Conclusion This study demonstrates the feasibility of performing antigen testing on buccal swab specimens from children with sickle cell disease as part of a newborn screening follow-up program with the aim of allowing specific unit matching to prevent alloimmunization with RBC transfusions. The general applicability of testing may be limited by a lack of uniform insurance coverage for buccal swab testing, however. *(J Pediatr 2014;165:1003-7)*.

Sickle cell disease (SCD) is a group of complex, chronic disorders characterized by unpredictable acute complications that can rapidly become life-threatening. Performed to treat or prevent certain complications of SCD, red blood cell (RBC) transfusions can prevent organ damage, reduce disease-associated mortality, increase blood oxygen-carrying capacity, and possibly alleviate or prevent other complications of the disorder. Most adult patients with SCD have received at least 1 RBC transfusion, and many have received multiple transfusions.[1]

Despite its efficacy in preventing and treating complications associated with SCD, transfusion therapy can be complicated by RBC alloimmunization, a common clinical sequelae of this treatment. A major contributor to the high rate of RBC alloimmunization in patients with SCD is the racial discordance between blood donors and recipients.[1,2] In the US, the majority of blood donors are of white/European descent, whereas most patients with SCD are of African descent. To date, efforts to prevent RBC alloimmunization have focused on prophylactic extended antigen testing, that is, testing and matching of antigens beyond the 6-11 antigens included in standard serologic testing. The goal of using the extended antigen panel is to identify molecularly matched donor units for the patient should a blood transfusion become necessary.[3]

National Institutes of Health guidelines recommend that individuals with SCD aged >6 months undergo RBC antigen phenotyping, that the individual's permanent record of RBC phenotyping be maintained in the transfusion service to optimize RBC antigen matching, and that a copy of the record be provided to the individual or family.[4]

In an attempt to increase the proportion of patients with SCD who undergo extended RBC antigen analysis during infancy, and to establish a practice that could be extended easily and economically to other programs, a pilot project using a noninvasive buccal swab sample and molecular test kit was initiated within the Indiana State Department of Health–supported newborn hemoglobinopathy screening follow-up program. The routine use of extended molecular RBC phenotyping could possibly identify individuals who may be at increased risk for alloimmunization and facilitate the use of optimally matched RBC components for patients with SCD, to decrease the rate of alloimmunization as well the risk of transfusion reactions, especially delayed transfusion reactions owing to existing alloantibodies.[5]

HEA	Human erythrocyte antigen
RBC	Red blood cell
SCD	Sickle cell disease

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Methods

Children with SCD were identified through Indiana's newborn screening program for SCD (Sickle SAFE Program: Screening, Assessment, Follow-up, and Education) and recruited by the Sickle SAFE program coordinator, an employee of the Indiana Hemophilia and Thrombosis Center. The Sickle SAFE Program provides education, referrals, and support to families in Indiana who have a child with a hemoglobinopathy. The Sickle SAFE program coordinator conducts a home visit with each family at the time of diagnosis and when the child is aged 12-18 months. All children enrolled in the Sickle SAFE Program between December 2011 and December 2013 were eligible to participate. The study protocol received Institutional Review Board approval. Informed consent was obtained from the parent or guardian of each study subject.

Buccal Epithelial Cell Sampling and Molecular Testing

Buccal epithelial cells were collected from each enrolled subject by scraping the inside of each cheek 6 times with 2 polyester- or cotton-tipped single-use sterile applicators. After discrepant results were found, owing to insufficient DNA collection, the number of swabs was increased from 2 to 4. DNA was extracted from the buccal swabs by either a manual or an automated method. Manual and automated DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Chatsworth, California), and automated extraction was done using the DNA purification from buccal swabs spin protocol on the QIAcube system (Qiagen).[6] Primary swabs were used as isolation and retention swabs for re-extraction, if necessary.

The isolated DNA was assayed using a bead array assay (HEA [human erythrocyte antigen] BeadChip; BioArray Solutions, Warren, New Jersey). The DNA was first amplified using corresponding primers in a multiplex polymerase chain reaction, as described previously.[7] The amplified DNA was elongated using elongation-mediated multiplex analysis of polymorphisms and then assembled onto the BioArray HEA BeadChip carrier.[7] The assay results were analyzed using the BioArray automated assay image acquisition system, and the subject's genotype and phenotype results were recorded. The HEA BeadChip assay tests for 33 blood group antigens, including antigens in the 11 blood group systems Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego, Colton, and Scianna.[8]

Results

One of the 93 individuals approached to enroll in this study declined to participate, preferring to delay testing until the child was older. Thus, a total of 92 children, ranging in age from 6 days to 2.8 years, were enrolled and tested between December 2011 and December 2013 (Table I). Eighty-seven subjects were of African descent, and 5 were of Hispanic descent. The majority (54.3%) had the HbSS form of SCD, and approximately one-third had the HbSC form. Study intake information identified 14 subjects who had undergone previous RBC antigen testing and 4 subjects who had received previous transfusions. Three of the 4 subjects who had received previous transfusions also had undergone previous serologic RBC phenotyping.

Table I. Subject demographic data and diagnoses (n = 92)

Characteristics	Value
Males/females, n	51/41
Age at molecular analysis, y, mean (SD)	0.71 (0.27)
Age, y, range	0.02-2.82
Race/origin, n (%)	
African American	66 (71.7)
West African	17 (18.5)
Central American	5 (5.4)
Central African	2 (2.2)
North African	1 (1.1)
Middle Eastern	1 (1.1)
Diagnosis, n (%)	
HbSS	50 (54.3)
HbSC	29 (31.5)
HbS β-thalassemia-+	5 (5.4)
HbS β-thalassemia-0	3 (3.3)
HbS α-thalassemia	3 (3.3)
β-thalassemia major	1 (1.1)
HbS-Osu Christianborg	1 (1.1)

Testing based on DNA extracted from the buccal swabs was successful overall, with extended RBC typing generated for all subjects. In a few cases (4 of 92), testing had to be repeated owing to discrepancies between molecular testing and serologic testing already on record, or because the testing demonstrated indeterminate calls for a few alleles. The results of analysis for 32 tested alleles in the 92 subjects are summarized in Table II.

Several instances of individuals with rare negative or rare positive phenotypes were detected. Rare negative phenotypes included subjects negative for MNS blood group antigen s (n = 5; 6.3%); Kell blood group antigen Js^b (n = 4; 4.3%); and Dombrock blood group antigens Do^b (n = 5; 5.4%), Jo^a (n = 3; 3.3%), and/or Hy (n = 2; 2.2%), including 2 subjects simultaneously negative for both Dombrock antigens Jo^a and Hy. Seven subjects (7.6%) were identified by molecular typing as likely having variants of the C antigen r's.

Fifteen subjects had been previously or subsequently phenotyped serologically. Molecular and serologic test results in these 15 subjects were generally comparable, although differences were detected in 3 cases (also mentioned above).

Discussion

Our findings illustrate the feasibility of using buccal swabs to facilitate a simple, noninvasive, molecular-based RBC antigen test to characterize an extended phenotype including more than 30 antigens in infants and young children with SCD. The results of our analysis of 32 alleles based on DNA