Predictors of Red Blood Cell Alloimmunization in a Brazilian Sickle Cell Disease (SCD) Cohort

Shannon Kelly[1,2], Liliana Preiss[3], Carla Dinardo[4], Maria do Carmo Valgueiro [5], Luciana Cayres[6], Shirley Lopes de Castilho[7], Yuelong Guo[3], Ester Sabino[8], Grier Page[3], Brian Custer[1]
for the NHBLI Recipient Epidemiology and Donor Evaluation (REDS)-III Study

Background

SCD patients form red blood cell (RBC) antibodies at higher rates than other transfused populations. Multiple predictors of alloimmunization have been reported but not well replicated in large SCD cohorts. We investigated the clinical, laboratory and genetic predictors of alloimmunization in the REDS-III Brazil SCD cohort.

Methods

- A large SCD cohort was established in Brazil to investigate transfusion outcomes. At participating sites, patients are currently transfused with ABO/D/Cc/Ee/Kell matched RBCs prophylactically and extended phenotypically matched RBCs after the first antibody forms. Policies for matching are center-specific and evolved to increased levels of matching over the period included in this study, lifetime exposure up to 2015.
- Transfused subjects with 1+ RBC alloantibody of defined specificity within the cohort were compared to transfused antibody negative subjects using chi squared to compare categorical variables and T-test or Wilcoxon rank-sum tests as appropriate to compare continuous variables. Multivariable logistic modeling was used to generate odds ratios (OR) and identify independent predictors of alloimmunization. P-values ≤0.05 for this analysis were considered significant.
- All subjects had peripheral blood whole genome SNP genotyping with a custom Affymetrix Axiom array (the Transfusion Medicine Array developed for REDS-III) with 879,00 SNPs that were imputed to 28,000,000 SNPs using 1000 Genomes cosmopolitan reference samples. GWA studies with the outcome of alloimmunization as a discrete trait were run using a logistic regression model (PLINK version 1.90a) adjusted for age, gender, transfusion history, clinical site and the first 10 eigen values for ancestry. A p value of $5x10^{-8}$ was considered significant.
- More clinically severe SCD genotypes (homozygous SS, $S\beta^0$ = S-beta thalassemia 0, SD-LA = SD-Los Angeles) and less clinically severe SCD genotypes ($S\beta^+$ = S-beta thalassemia+, SHPFH = S-Hereditary persistence of fetal hemoglobin, SKW = S-K Woolwich) were grouped together in all analyses.

Results

Of the 2795 cohort patients, 2272 (81.3%) transfused subjects were included in this analysis. There were 129 alloimmunized children <18 years (11.0% of 1172) and 224 alloimmunized adults (20.4% of 1100).

Figure 1: Red Blood Cell Antibodies Formed in the REDS-III SCD Cohort

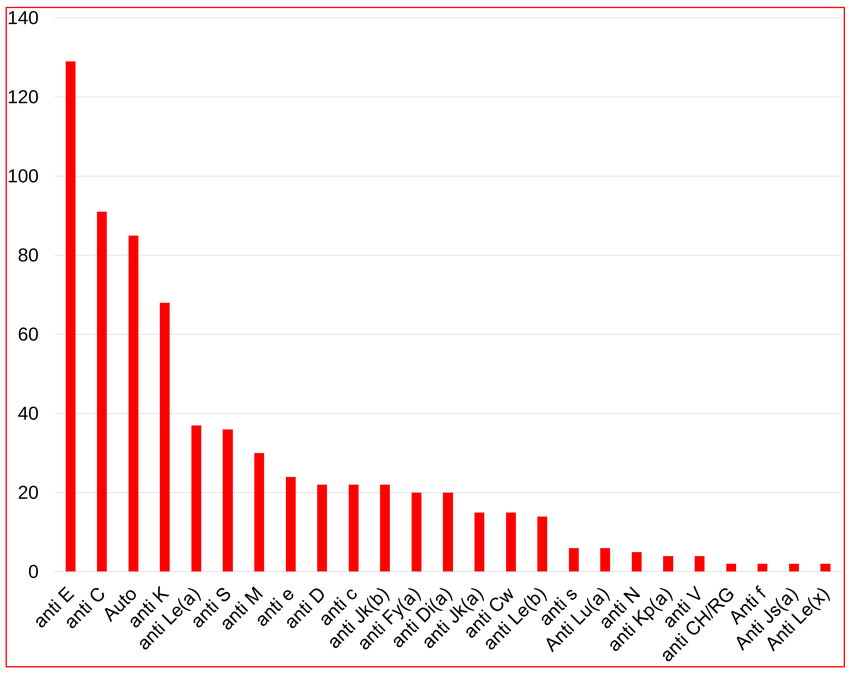


Table 1: Proportion of Children and Adults with 1, 2 or 3+ RBC Antibodies

	Children			Adults		
SCD type	1 Antibody	2 Antibodies	3+ Antibodies	1 Antibody	2 Antibodies	3+ Antibodies
SS/$S\beta^0$/SD-LA	75 (7.5%)	32 (3.2%)	16 (1.6%)	96 (10.4%)	62 (6.7%)	40 (4.3%)
SC/$S\beta^+$ /SHPFH/SKW	5 (2.9%)	0	1 (0.6%)	18 (10.3%)	6 (3.4%)	2 (1.1%)
Total	80 (6.8%)	32 (2.7%)	17 (1.5%)	114 (10.4%)	68 (6.2%)	42 (3.8%)

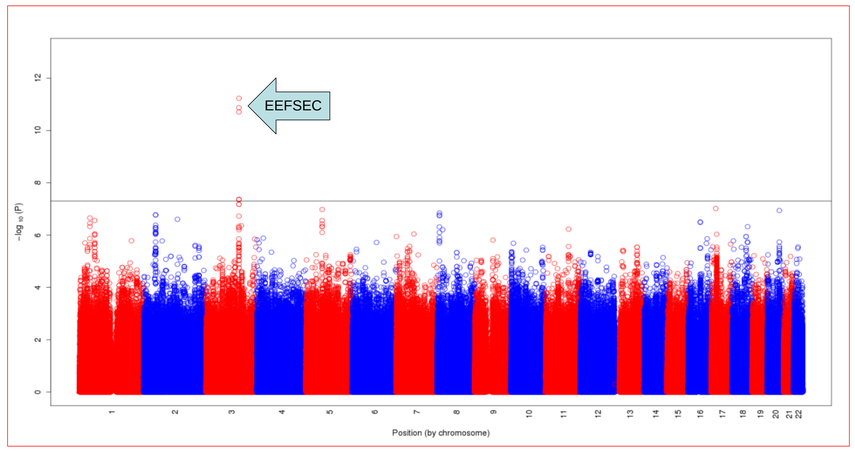
Table 2: Comparison of Antibody Positive and Negative REDS-III Cohort Patients

	Alloimmunized		Not Alloimmunized			
	N or Median	% of Row or Range	N or Median	% of Row or Range	Total	P value
SCD type						
SS/$S\beta^0$/SD-LA	321	16.7%	1,604	83.3%	1,925	0.0004
SC/$S\beta^+$ /SHPFH/SKW	32	9.2%	315	90.8%	347	
Age						
<5	5	3.9%	122	96.1%	127	<0.0001
5<10	28	7.6%	341	92.4%	369	
10<20	111	14.2%	669	85.8%	780	
20<30	91	20.9%	345	79.1%	436	
30<50	99	21.2%	369	78.8%	468	
50+	19	20.7%	73	79.3%	92	
Gender						
Female	213	17.7%	992	82.3%	1,205	0.003
Male	140	13.1%	927	86.9%	1,067	
Transfusion History						
1-5	70	8.4%	761	91.6%	831	<0.0001
6-10	49	12.5%	344	87.5%	393	
11-20	55	18.2%	248	81.8%	303	
21-40	50	23.4%	164	76.6%	214	
41-60	26	28.6%	65	71.4%	91	
61-80	15	34.9%	28	65.1%	43	
81+	64	35.6%	116	64.4%	180	
Missing	24	6.8%	193	10.1%	217	
Clinical Site / Brazilian City						
Hemominas – Belo Horizonte	87	14.10%	530	85.90%	617	0.002
Hemominas – Juiz de Fora	27	13.70%	170	86.30%	197	
Hemominas – Montes Claros	24	9.60%	225	90.40%	249	
Hemope - Recife	72	14.80%	416	85.20%	488	
Hemorio – Rio de Janeiro	123	19.70%	501	80.30%	624	
Institute of Childhood Cancer (ITACI) – Sao Paulo	20	20.60%	77	79.40%	97	
Presence of Auto Antibody						
Yes	46	61.3%	29	38.7%	75	<0.0001
No	307	14.0%	1,890	86.0%	2,197	
Transfused at outside institution						
Yes	60	16.0%	314	84.0%	374	0.7
No	288	15.3%	1,590	84.7%	1,878	
Age at first transfusion (dichotomized age 10)						
<10	206	14.7%	1,196	85.3%	1,402	0.2
10+	60	17.2%	289	82.8%	349	
Splenectomy						
Yes	51	16.2%	263	83.8%	314	0.7
No	301	15.4%	1,656	84.6%	1,957	
Ferritin (ng/mL)	515.0	(17-12,656)	277.0	(1-9,676)		<0.0001
Lactate Dehydrogenase (LDH) (U/L)	569.0	(159-2,988)	507.0	(2-3,149)		0.048
Chronic pain						
Yes	61	20.6%	235	79.4%	296	0.01
No	292	14.8%	1,683	85.2%	1,975	
Leg ulcers						
Yes	55	24.6%	169	75.4%	224	<0.0001
No	297	14.6%	1,742	85.4%	2,039	
Avascular Necrosis						
Yes	36	16.3%	185	83.7%	221	0.7
No	314	15.4%	1,727	84.6%	2,041	
Priapism (males only)						
Yes	24	14.8%	138	85.2%	162	0.5
No	114	12.9%	772	87.1%	886	
Comorbid Auto-immune disorder						
Yes	26	43.3%	34	56.7%	60	<0.0001
No	327	14.8%	1,885	85.2%	2,212	

In multivariable models, the following variables were identified as independent predictors of alloimmunization:

- **Age (OR 4.2, p=0.009, for age 50+ years compared to 0-4 years)**
- **Transfusion history (OR 3.5, p<0.0001, for 81+ lifetime transfusions compared to 1-5)**
- **Clinical site (OR 2.3, p<0.005 for the Sao Paulo site compared to the Belo Horizonte site)**
- **Gender (OR 1.3, p=0.04, for females compared to males)**
- **Hemolysis (OR 1.3, p=0.05, for log transformed LDH)**
- **Presence of autoimmune disorders (OR 4.5, p<0.0001)**

Figure 2: Manhattan Plot Showing Genome Wide $-\log_{10}$ P Values for Association of SNPs with Alloimmunization



Genome wide analysis identified a single locus with several SNPs that was associated with alloimmunization in a model controlling for age, gender, transfusion burden and clinical site - Eukaryotic Elongation Factor, Selenocysteine-TRNA Specific (EEFSEC). This gene is responsible for incorporation of selenocysteine into proteins. Due to the lower reduction potential of selenocysteine compared to cysteine, selenoproteins typically function to maintain a reducing environment within cells.

Conclusions

- Alloimmunization was driven by age, gender, clinical site and transfusion burden in the REDS-III Brazil Sickle Cell Disease Cohort.
- Independent predictors of alloimmunization identified in other cohorts such as age at first transfusion, chronic pain and avascular necrosis were not replicated in this analysis
- Novel findings include:
  - Hemolysis remained significantly associated with alloimmunization after controlling for lifetime transfusions.
  - Systemic immune dysregulation may be present in SCD patients who develop RBC alloantibodies as auto immune disorders were more common in alloimmunized.
  - GWA identified one locus, EEFSEC, associated with alloimmunization. Further studies to validate this finding in other cohorts, explore the mechanistic link with alloimmunization as well investigate genetic predictors of specific alloantibodies and alloimmunization in homozygous SS patients within the REDS-III SCD cohort are currently underway.

1 Blood Systems Research Institute, San Francisco CA
2 UCSF Benioff Children's Hospital Oakland, Oakland CA
3 Research Triangle Institute, Rockville, MD
4 Fundacao Pro Sangue, Sao Paulo, Brazil
5 Fundacao Hemope, Recife, Brazil
6 Fundacao Hemominas, Belo Horizonte, Brazil
7 Fundacao Hemorio, Rio de Janeiro, Brazil
8 University of Sao Paulo, Sao Paulo, Brazil