GWAS of Osmotic Hemolysis in 12,352 Healthy Blood Donors Identifies Rare and Common Red Cell Genetic Variants that Modulate Steady State Hemolysis in Patients With Sickle Cell Disease

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Introduction

Hemolysis is important in transfusion medicine

In the US blood is often stored before use and over time the blood begins to hemolyze. There is a extensive inter-individual variation in the numerous hemolysis rates. As a result the US FDA have set guidelines for the maximal allowable hemolysis and storage time for a unit to be used in transfusion. The focus on this study is to understand the genetic and non-genetic factors which affect of measure of hemolysis, osmotic fragility (pink) test.

Hemolysis in sickle cell patients

Sickle cell disease is a hemolytic anemia with variable, but often severe intravascular hemolysis being a hallmark of the disease. Some studies have found an association between the degree of hemolysis and survival in sickle cell patients.

Osmotic fragility test (Pink test)

There are several in vitro hemolysis measures. One is the osmotic fragility test of red blood cells is a test that has been used in diagnosis of spherocytosis. It is a composite index of red blood cells shape, hydration, and proneness to *in vivo* destruction within limitations.

High throughput genome wide screening

We hypothesized that a high throughput screen of red blood cell susceptibility to osmotic hemolysis, linked to GWAS, would identify rare and common genetic variants that would modulate cellular structure and function during red blood cell storage and in hemolytic diseases like sickle cell and malaria.

Methods

Study population

The NHLBI Recipient Epidemiology and Donor evaluation Study III (REDS-III) Red Blood Cell Omics (RBC-Omics) study enrolled 13,403 successful blood donors from 4 blood centers in the United States. Two labs were used for red blood cell phenotyping of leukoreduced RBC unit-derived samples stored for ~42 days under blood bank conditions. Red blood cell osmotic fragility was assayed in 12,352 donors, including 1,483 African American, 1,477 Asian and 960 Hispanic donors.

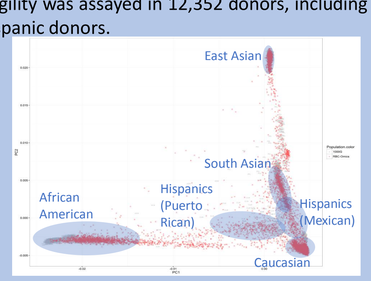

Figure 1. The racially diverse study population. The top two principle components (PC) of the study population with RBCOmics participants shown in red and 1000 genome participants shown in gray.

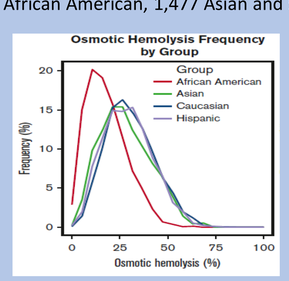

Figure 2. Effect of self reported race on Osmotic hemolysis. (Kanias et al 2017)

References

Kanias T et al l. "Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-Omics study" 2017 Jun 27;1(15):1132-1141

REDS-III study

The NHLBI Recipient Epidemiology Donor Evaluation Study-III (REDS-III), Red Blood Cell (RBC)-Omics Study, is the responsibility of the following persons: Hubs: A. E. Mast, J. L. Gottschall, W. Bialkowski, L. Anderson, J. Miller, A. Hall, Z. Udee, and V. Johnson, BloodCenter of Wisconsin, Milwaukee, WI; D. J. Triulzi, J. E. Kiss, and P. A. D'Andrea, The Institute for Transfusion Medicine (ITXM), Pittsburgh, PA; E. L. Murphy and A. M. Guiltinan, University of California, San Francisco, San Francisco, CA; R. G. Cable, B. R. Spencer, and S. T. Johnson, American Red Cross Blood Services, Farmington, CT; Data coordinating center: D. J. Brambilla, M. T. Sullivan, S. M. Endres, G. P. Page, Y. Guo, N. Haywood, D. Ringer, and B. C. Siege, RTI International, Rockville, MD; Central and testing laboratories: M. P. Busch, M. C. Lanteri, M. Stone, and S. Keating, Blood Systems Research Institute, San Francisco, CA; T. Kanias and M. Gladwin, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; Steering committee chairman: S. H. Kleinman, University of British Columbia, Victoria, BC, Canada; National Heart, Lung, and Blood Institute, National Institutes of Health: S. A. Glynn, K. B. Malkin, and A. M. Cristman.

Results

Genotyping and imputation

Genotyped using a custom Affymetrix Axiom Transfusion Medicine Array (TM-Array), which contained approximately 875,000 SNPs enriched for blood and transfusion related polymorphisms. Imputation was conducted using 1000 genome phase 3 participants as the reference panel. Association analyses was conducted by adjusting demographic covariates and the top 10 principle components to account for population substructure.

Genome wide association in the complete RBC-omics data set revealed 18 genes/loci to be associated with osmotic hemolysis at a genome wide significance threshold of $P<5x10^{-8}$ in the entire dataset. The genes included candidates with clear internal validity such as *HBB* (specifically rs344 the HbS variant) and promotor regions of *HBA2* (thalassemic variants), as well as SNPs in logical candidates, such as Ankyrin, Spectrin, and Aquaporin 1, mutations in which are responsible for spherocytosis and red cell membrane water transport defects, among others.

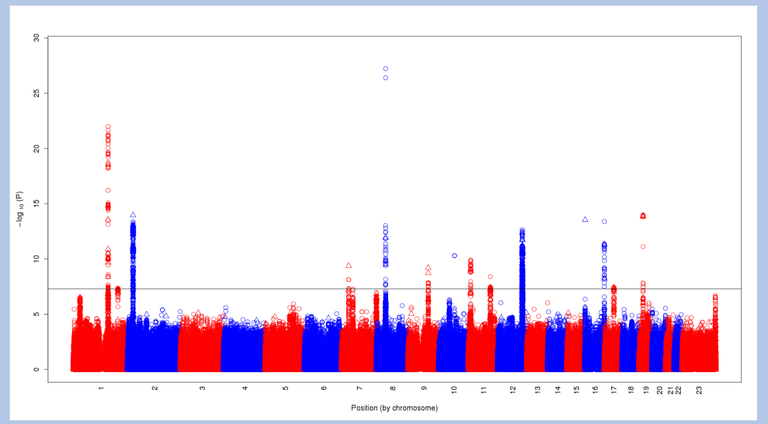

Figure 3. Manhattan plot for osmotic hemolysis among all RBC-Omics participants.

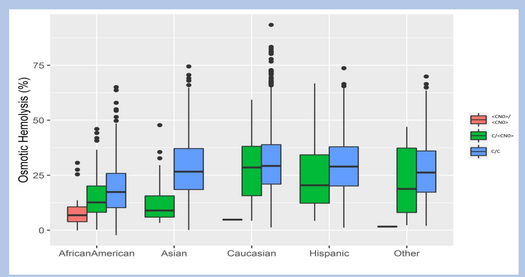

Figure 4. Alpha thalassemia deletion is associated with decreased osmotic hemolysis.

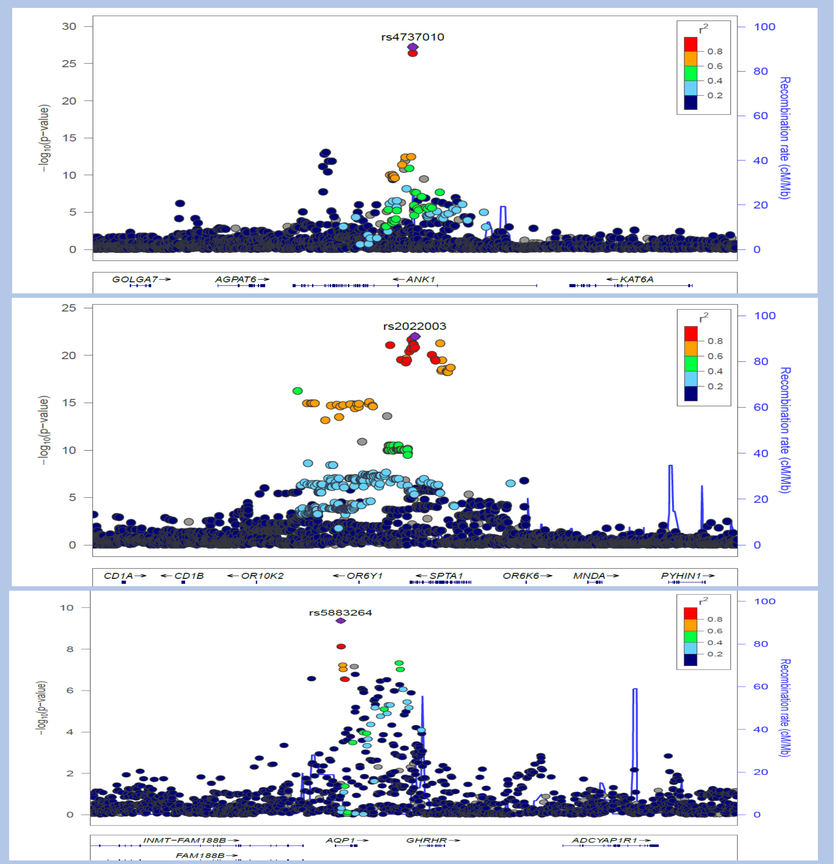

Figure 5. LocusZoom plot for ANK1, SPTA1, and AQP1 gene regions. LD estimated in 1000G EUR.

To validate the potential relevance of these finding to human disease, we evaluated the top 100 regions found in the RBC-Omics studies in the 852 pediatric and adult patients with sickle cell disease enrolled in the PUSH and Walk-PHASST screening studies with genome SNP data, and analyzed the association of the SNP with the intensity of steady state hemolysis, as measured by a principle component analysis of reticulocyte count, bilirubin, lactate dehydrogenase and aspartate amino transferase. After multiple testing correction, numerous genes were replicable associated with the intensity of hemolysis, including previously identified, and novel, thalassemia and ankyrin/spectrin mutations, SNPs in red cell metabolism enzymes genes (*HK1, TKTL1 MTOR signaling*), ion channels (*PIEZO1*), as well as regulatory gene (*ZNF573*) and less obvious genes (*FLN, SBF2, GAB3*, and *TMEM116*).

Conclusions

The first genome wide association study of the genetic variability underlying human red blood cell susceptibility to osmotic stress hemolysis identified a number of candidate genes for the regulation of the degree red blood cell hemolysis that were also found to affect hemolysis in a Sickle Cell Diseases cohort. We hypothesize that many of these candidate SNPs will modulate the severity of red cell hemolysis in other diseases like malaria and potential outcomes after routine red blood cell storage and transfusion. These studies highlight the discovery of a number of new thalassemia, metabolic and ion/water transport variants that modulate the severity of steady state hemolysis under disease stress.

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Conflict of interest disclosure

Alan E. Mast received research funding from Novo Nordisk and has received honoraria from Siemens. The remaining authors declare no competing financial interests.