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

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 that 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 cell storage and in hemolytic diseases like sickle cell and malaria.

Methods

Study population

The NHBLI Recipient Epidemiology and Donor evaluation Study III (REDS-III) Red Blood Cell Genomics (RBC-Gomics) study enrolled 33,403 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 and 960 Hispanic donors.

The racially diverse study population. The top two principle components of the study population with RBC-Gomics participants shown in red and 1000 genome participants shown in gray.

Figure 1. The racially diverse study population. The top two principal components of the study population with RBC-Gomics 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)

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

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

References

Kanias T et al 1. “Ethnicity, sex, and age are determinants of red cell storage and cell hemolysis: results of the REDS-III RBC-Gomics study” 2017 Jun 27;21(15):1332-1141

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 cell blood storage and transfusion. These studies highlight the discovery of a number of new thalassemia, metabolic and ion/lwater transport variants that modulate the severity of steady state hemolysis under disease stress.

Acknowledgements

We express our sincere thanks to the REDS-III participants who contribute to these studies. This study was supported by the National Institutes of Health, National Heart, Lung, and Blood Institute (NHLBI) contracts: HHSN2682011-00031I, HHSN2682011-00032I, HHSN2682011-00033I, HHSN2682011-00044I, HHSN2682011-00051I, HHSN2682011-00065I, HHSN2682011-00077, HHSN2682011-00086I, and HHSN2682011-00090I.

Conflict of interest disclosure

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