NHLBI REDS-III PROGRAM
RBC-OMICS STUDY

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Blood Systems Research Institute
University of California, San Francisco
PI, REDS-III Central Laboratory
History of the REDS programs (NHLBI)

Goal of the REDS research program: improve the safety and availability of transfused blood products in the U.S. and internationally

The National Heart, Lung, and Blood Institute Recipient Epidemiology and Donor Evaluation Study (REDS-III): a research program striving to improve blood donor and transfusion recipient outcomes

Steven Kleinman,1 Michael P. Busch,23 Edward L. Murphy,24 Hua Shan,2 Paul Ness,52 and Simone A. Glynn6 for The National Heart, Lung, and Blood Institute Recipient Epidemiology and Donor Evaluation Study (REDS-III)
Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)

National Heart Lung and Blood Institute (NHLBI)

Data coordinating center
RTI international

Blood Center of Wisconsin
Instit. for Tx Medicine
UCSF/BCP
Yale/ARC

Central Laboratory BSRI

Brazil
South Africa
China

Establish linked donor-component-recipient outcomes database

Prospective collections
- DENV/CHIKV/ZIKB
- Sickle cell disease
- Infectious markers (HIV)
- HIV/HBV
- Obstetric hemorrhage
- SFTSV
- HIV/HBV (genotyping)

Pre-existing collections
- FastTrack studies:
  1. Alloimmunization study
  2. GBV-C study
  3. TMPRSS6 study
  4. HEIRS

Prospective collections
- Larger studies:
  1. RBC omics
  2. STRIPE
  3. RETRO
  4. CHILL

Closure:
- Analyses
- Presentations
- Papers

ZIKV studies

Phase 1 (2011)
- FastTrack studies:
  1. Alloimmunization study
  2. GBV-C study
  3. TMPRSS6 study
  4. HEIRS

Phase 2 (2013)
- Larger studies:
  1. RBC omics
  2. STRIPE
  3. RETRO
  4. CHILL

Phase 3 (2018-2020)
- Closure:
  - Analyses
  - Presentations
  - Papers

Brazil
- DENV/CHIKV/ZIKB
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- Obstetric hemorrhage
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South Africa
- DENV/CHIKV/ZIKB
- Sickle cell disease
- Infectious markers (HIV)
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- Obstetric hemorrhage
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China
- DENV/CHIKV/ZIKB
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Pre-existing collections

Prospective collections

Establish linked donor-component-recipient outcomes database

ZIKV studies
Covers the time period from 6/2012 – 12/2016 (donors)
1/2013 – 12/2016 (recipients)

Unique linking code is the DIN + Product Code

Expected scope of completed dataset:
Detailed data on donors, donations
  25.5 million records
Components and component modifications
  8.2 million records
Recipients (inpatient and outpatient included)
  672 million records
◊ Overall descriptive analysis of blood recipients and survival in hospital

◊ Effect of donor age, sex and prior donation intensity on blood transfusion recipient mortality and other outcomes

◊ RBC Alloimmunization (collected operational antibody and antigen data for donor and/or recipients)

◊ Impact of anti-coagulant use [comparison of outcomes for patient on/not on direct oral anti-coagulation (DOACs) therapy]

◊ Blood transfusion and the risk for thrombosis
REDs-III Transfusion Medicine Array Content Design

Affymetrix Axiom genome wide SNP array

- Standard coverage to MAF > 5%
  - 348K SNPs
- Addition East Asian Coverage
  - 48K SNPs
- Addition AA Coverage
  - 163K SNPs
- Customized for this array

SNPs we selected
- Restless Legs
  - 13 SNPs
- Transplant
  - SNPs
  - 145 SNPs
- SNPs in blood and iron disorder gene
  - 3885 SNPs
- GWAS catalog blood and iron transport
  - 1164 SNPs
- Blood Grp Genes
  - 729 SNPs
- Brazil SNPs
  - 238 SNPs
- AA SNPs
  - 41 SNPs
- SNPs in blood and iron transport
  - ~34,000 SNPs
- RH c d e and Globin CNP
  - 1000 CNPs

CNPs we selected
- Y and Mt genes
  - 1200 markers
- Nonsynonymous SNPs
  - < 80K SNPs
- ADME
  - 2K SNPs
- eQTL markers
  - Affy and GTEX
  - 23K SNPs
- HLA/KIR
  - 14K SNPs

SNPs we selected
- Platelets
  - 3856 Genes
- Cytokine
  - 238 Genes
- TGF Genes
  - 155 Genes
- SCD Genes
  - 48 Genes
- RBCs
  - 1285 Genes
- Nearby genes
  - 936 genes
- Y and Mt genes
  - 141 Genes
- 879 K SNP and CNPs

Genes we selected
- Platelets
  - 3856 Genes
- Cytokine
  - 238 Genes
- TGF Genes
  - 155 Genes
- SCD Genes
  - 48 Genes
- RBCs
  - 1285 Genes
- Nearby genes
  - 936 genes
- Y and Mt genes
  - 141 Genes

Affymetrix Content
- ADME
  - 2K SNPs
- eQTL markers
  - Affy and GTEX
  - 23K SNPs
- HLA/KIR
  - 14K SNPs
- Y and Mt genes
  - 1200 markers
- Nonsynonymous SNPs
  - < 80K SNPs
Donor genetic variations can affect hemolytic propensity and RBC storage recovery and function

Karias and Gladwin, Transfusion 2014
Genetic polymorphisms linked to susceptibility to malaria

Adel Driss\textsuperscript{1}*, Jacqueline M Hibbert\textsuperscript{1}, Nana O Wilson\textsuperscript{1}, Shareen A Iqbal\textsuperscript{2}, Thomas V Adamkiewicz\textsuperscript{3} and Jonathan K Stiles\textsuperscript{1}*

\textbf{Table 1 Genetic mutations involved in susceptibility/resistance to \textit{P. falciparum} malaria}

<table>
<thead>
<tr>
<th>Gene (\textit{Symbol})</th>
<th>Phenotype</th>
<th>Proposed protective mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin C (HbC)</td>
<td>↓UM &amp; ↓SM</td>
<td>Reduced cyto-adherence of infected erythrocytes</td>
<td>[29,47]</td>
</tr>
<tr>
<td></td>
<td>↓SM, ↓parasitaemia</td>
<td>Reduced erythrocyte invasion by merozoites, lower intra-erythrocytic parasite growth, and enhanced phagocytosis of infected erythrocytes.</td>
<td>[48,49]</td>
</tr>
<tr>
<td>Haemoglobin E (HbE)</td>
<td>↓SM</td>
<td>Selective sickling of infected sickle trait erythrocytes leading to enhanced clearance by the spleen. Reduced erythrocyte invasion, early phagocytosis, and inhibited parasite growth under oxygen stress in venous micro vessels. Enhancement of innate and acquired immunity.</td>
<td>[7,50]</td>
</tr>
<tr>
<td>α-thalassaemia (α-thal)</td>
<td>↓SM &amp; ↓SMA</td>
<td>Reduced resetting. Increased micro-erythrocyte count in homozygotes reduces the amount of haemoglobin lost for given parasite density, thus protecting against severe anaemia.</td>
<td>[51-53]</td>
</tr>
<tr>
<td>β-thalassaemia (β-thal)</td>
<td>↓SM</td>
<td></td>
<td>[54,55]</td>
</tr>
<tr>
<td>Glucose-6-Phosphate dehydrogenase (G6PD)</td>
<td>↓UM &amp; ↓SM</td>
<td>Increased vulnerability of the G6PD deficient erythrocyte to oxidant stress causes its protection against parasitization.</td>
<td>[56-59]</td>
</tr>
<tr>
<td>Pyruvate kinase (PKLR)</td>
<td>↓parasitaemia</td>
<td>Invasion defect of erythrocytes and preferential macrophage clearance of ring-stage-infected erythrocytes.</td>
<td>[60]</td>
</tr>
<tr>
<td>Ovalocytosis (SLC4A1)</td>
<td>↓SM &amp; ↓CM</td>
<td>Inhibition of merozoite entry into the red cell, impairment of intracellular parasite growth and prevention of the erythrocyte lysis that occurs with parasite maturation, leading to release of merozoites into the blood stream.</td>
<td>[61,62]</td>
</tr>
<tr>
<td>Elliptocytosis</td>
<td>↓SM</td>
<td></td>
<td>[63]</td>
</tr>
<tr>
<td>Glycophorins A (GYP ABC)</td>
<td>↓SM</td>
<td></td>
<td>[64,65]</td>
</tr>
<tr>
<td>Blood Groups (ABO)</td>
<td>↓SM</td>
<td>Reduced \textit{P. falciparum} rosetting.</td>
<td>[66-68]</td>
</tr>
<tr>
<td>Haptoglobin (HP)</td>
<td>↓SM</td>
<td>Oxidative damage to uninfected cells might be more marked in HP polymorphic individuals since HP proteins bind less efficiently to Hb, increasing premature destruction of erythrocytes and stimulating cytokine release by these circulating cells.</td>
<td>[69-71]</td>
</tr>
<tr>
<td>Nitric oxide synthase 2 (NOS2)</td>
<td>↓SM</td>
<td>Increased NO production induces Th1 cytokines which activate macrophages and could thus be an anti-malarial resistance mechanism.</td>
<td>[72,73]</td>
</tr>
<tr>
<td>haem oxygenase I (HO-I)</td>
<td>↓CM</td>
<td>Release of free haem in the blood stream.</td>
<td>[13,14]</td>
</tr>
</tbody>
</table>
### Polymorphisms in hemoglobins, red cell membrane proteins and red cell enzymes

<table>
<thead>
<tr>
<th>Category</th>
<th>Disease</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme Defects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyruvate Kinase deficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G6PD deficiency</td>
<td>Multiple polymorphisms described.</td>
</tr>
<tr>
<td><strong>Membrane Defects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary Ovalocytosis</td>
<td>27 base pair deletion in band 3</td>
<td></td>
</tr>
<tr>
<td>Hereditary Eliptocytosis (HE)</td>
<td>Multiple genes- Spectrin, Protein 4.1, Pallidin, Band 3</td>
<td></td>
</tr>
<tr>
<td>Hereditary Spherocytosis</td>
<td>Multiple genes - Spectrin, Ankyrin, Band 3, protein 4.2</td>
<td></td>
</tr>
<tr>
<td>Ankyrin</td>
<td>Hereditary Spherocytosis (HS)</td>
<td>Dominant HS - ANK1 gene</td>
</tr>
<tr>
<td></td>
<td>Hereditary Spherocytosis</td>
<td></td>
</tr>
<tr>
<td>Band 3</td>
<td>South east asian Ovalocytosis</td>
<td>SLC4A gene - multiple polymorphisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 base pair deletion in band 3</td>
</tr>
<tr>
<td>Beta Spectrin</td>
<td>Hereditary Eliptocytosis</td>
<td>SPTB gene, Dominant pattern</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SPTB gene, Dominant pattern</td>
</tr>
<tr>
<td></td>
<td>Hereditary Eliptocytosis (most common HE)</td>
<td></td>
</tr>
<tr>
<td>Alpha Spectrin</td>
<td>Hereditary Spherocytosis</td>
<td>SPTA gene, dominant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SPTA 1 gene , Recessive pattern</td>
</tr>
<tr>
<td></td>
<td>Hereditary Spherocytosis (in japanese)</td>
<td></td>
</tr>
<tr>
<td>Protein 4.2</td>
<td></td>
<td>Recessive</td>
</tr>
<tr>
<td></td>
<td>Hereditary Eliptocytosis</td>
<td></td>
</tr>
<tr>
<td>Protein 4.1</td>
<td></td>
<td>EPB41 gene</td>
</tr>
<tr>
<td><strong>Globin Defects</strong></td>
<td>alpha thalasemias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>beta thalasemias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemaglobin S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemaglobin C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemaglobin E</td>
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</table>
Omics in the Literature

What does analysis of the last 15 years of literature on the different omics tell us about the growing importance of the field, and the move towards a more integrated approach? PubMed was searched for “transcriptomics,” “genomics,” “metabolomics,” “proteomics” and “integrated omics” with a date filter of 2001 to 2015. The data were then analyzed in Microsoft Excel 2013.
Harness advances in “Omics” technologies and system biology approaches to:

- Further our understanding of RBC biology
- Inform efforts to develop transfusion products from stem cells or their progenitors
- Better understand the effect of processing, storage conditions and donor variability
- Evaluate correlations between “what’s in the bag” and 24h in vivo recovery evaluations and other measures of RBC effectiveness
- Evaluate novel additive solutions or storage strategies
The REDS-III RBC-Omics Study aims to identify novel polymorphisms in hemoglobin and iron regulatory proteins, understand the genetic and metabolic basis for donor-specific differences in storage hemolysis, and establish a sharable biorepository of RBC, plasma, WBC, and DNA samples for BioLINCC enrollment.

**A- Screening phase:**
- Hemolysis phenotype on 14,000 RBC donors
- Screening for extreme hemolyzers

**B- GWAS on white blood cells from 14,000 donations**

**C- Recall phase:**
- Stability at 2 time-points & over 3 times during storage
- Confirm selection of extreme hemolyzers

**D- Metabolomics on 200 extreme hemolyzers**

This project pursues the overarching hypothesis that genetic variation in donors underlies the variable propensity of erythrocytes to hemolyze during routine RBC storage and after transfusion.
Components retrieved

- LR-Filter
  - Recovery of WBC Aliquots WBC/DNA

- 12.5mL transfer bag kept for storage

- LR-pRBC Component

- 12.5mL transfer bag kept for storage

Testing

DNA extraction for GWAS
- Affymetrix
  - To identify SNPs associated with RBC function and characteristics

Hemolysis assays:

- Screening:
  - Storage hemolysis (spontaneous)
  - Oxidative hemolysis (peroxidation)
  - Osmotic fragility (hypotonic shock)

- Selection of extremes to enrich for genetic variants with larger functional effect

Recall:

- Storage hemolysis
- Oxidative hemolysis
- Osmotic fragility
- Mechanical fragility
  - Confirm reproducibility
  - Changes overtime of storage
Storage phenotype hemolysis assays

- RBC donations evaluated for hemolysis on a representative 15 mL transfer bag, which was stored for 39-42 days (1-6°C)
Characterization of donor predisposition to hemolysis

Testing and Analysis:
Effect of donor’s sex, age, and donation history on:
- Storage hemolysis (n=10,584); Osmotic hemolysis (n=10,624)
- Oxidative hemolysis (n=8533)

Kanias et al. Blood Advances, 2017
Donor age is associated with changes in predisposition to storage hemolysis in a sex-specific manner.

Sex and age differences in osmotic hemolysis

Age and sex differences in osmotic fragility are largest during reproductive years

Kanias et al. Blood Advances, 2017
Sex and age differences in oxidative hemolysis

➢ Aging in both sexes is associated with increased resistance to AAPH-induced oxidative hemolysis

Kanias et al. Blood Advances, 2017
Effect of donor race-ethnicity on hemolysis

Kanias et al. Blood Advances, 2017
The 3 hemolytic end points poorly correlate with each other

<table>
<thead>
<tr>
<th></th>
<th>Pearson’s $r$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage vs. osmotic</td>
<td>0.103</td>
<td>2.2x10^{-16}</td>
</tr>
<tr>
<td>Storage vs. oxidative</td>
<td>0.019</td>
<td>0.09</td>
</tr>
<tr>
<td>Osmotic vs. oxidative</td>
<td>0.048</td>
<td>6.6x10^{-6}</td>
</tr>
</tbody>
</table>

➢ The poor correlation among the hemolytic tests may result from different genetic pathways unique to each stress.
Association between donation intensity and oxidative hemolysis or plasma ferritin

- Both parameters are inversely correlated with the number of donations
Identifying extreme hemolyzers during screening – Reproducibility at recall

Storage Hemolysis

Osmotic Hemolysis

Oxidative Hemolysis

To identify extreme hemolyzers <5\textsuperscript{th} and >95\textsuperscript{th} percentile of the hemolysis assay

→ GWAS and Metabolomics

Intra-assay correlations for screening vs recall results in transfer bags

Storage Hemolysis

Osmotic Hemolysis

Oxidative Hemolysis

Correlation: 0.30

Correlation: 0.87

Correlation: 0.54
Hemolysis parameters over time of storage

By site

By race/intensity

By gender

Days of storage

Spontaneous hemolysis

Influenced by processing methods

Oxidative hemolysis

Osmotic hemolysis

Mechanical fragility

Driven by testing lab differences

Most Asian are coming from BCP

With reproducible assays

we clearly identified some differences in hemolysis phenotypes associated with race/ethnicity and gender

→ potentially driven by underlying genes

waiting for the GWAS study...

Clear race/ethnicity differences

Clear gender differences

High intensity donation impacts RBC storage?
Design of REDS-III
“Transfusion Medicine Array”

• Formed expert groups
  • Blood groups
  • SCD experts
  • Transplantation
  • Iron metabolism
  • RBC Metabolism
  • Immunology/HLA
  • Coagulation
  • Interval Study

• Bioinformatics
  • Pulled existing lists
  • Mining of repositories
  • Added content from other studies
  • Chagas GWAS data
  • Brazilian Sequencing data
Affymetrix Axiom genome wide SNP array

- Standard coverage to MAF > 5%
  - 348K SNPs
- Addition East Asian Coverage
  - 48K SNPs
- Addition AA Coverage
  - 163K SNPs
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  - 13 SNPs
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CNPS we selected:
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SNPs we selected to 1% MAF:
- Iron Genes
  - 141 Genes
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  - 238 Genes
- TGF Genes
  - 155 Genes
- Nearby genes
  - 936 genes
- SCD Genes
  - 48 Genes
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- ADME
  - 2K SNPs
- eQTL markers
  - Affy and GTEX
  - 23K SNPs
- HLA/KIR
  - 14K SNPs
Genetic Ancestry using TM-Array

- East Asians
- Hispanics
- African
- Caucasians
"It is quite unusual to have 14 genome wide significant hits, of which numerous make high biological sense, in a GWA study with a relatively modest sample size."
Osmotic hemolysis: GWA interpretation

• This was a analysis with all subjects together (Asian, African American, Caucasian, Hispanics. N = 12,352

• ANK1 (Ankyrin 1; most significant SNP p = 6E-28). Mutations in erythrocytic ankyrin 1 associated with half of all patients with hereditary spherocytosis.

• Best SNP also seen in Chen P et al. Multiple nonglycemic genomic loci are newly associated with blood level of glycated hemoglobin in East Asians. Diabetes. 2014-03-19

• SPTA1 (Spectrin Alpha, Erythrocytic 1; most significant SNP p = 1E-22). Mutations in this gene cause Elliptocytosis-2, Pyropoikilocytosis, and Spherocytosis, type 3.

• ATAD2B/KLHL29 (most significant SNP p = 1E-14).

• AQP1 (Aquaporin 1/Colton Blood Group; most significant indel p = 4E-10). This gene encodes a small integral membrane protein with six bilayer spanning domains that functions as a water channel protein.
Osmotic hemolysis: GWA interpretation

- HK1 (Hexokinase 1; most significant SNP p = 5E-11). This gene encodes a ubiquitous hexokinase which localizes to the outer membrane of mitochondria. Mutations associated with hemolytic anemia due to hexokinase deficiency.
- SWAP70 (SWAP Switching B-Cell Complex Subunit 70; most significant SNP p=1.3E-10).
- MYO9B (Myosin IXB; most significant SNP p = 1E-14)
- ATP2B4 (ATPase plasma membrane Ca2+ transporting 4; most significant SNP p = 4.5E-08, borderline GW significant). These enzymes critical role in intracellular calcium homeostasis.
- CHR 12 hit is broad: SH2B3, ATXN2, BRAP, **ACAD10, ALDH2**, MAPKAPK5, ADAM1A, TMEM116, ERP29, NAA25, TRAFD1, HECTD4, RPL6
Osmotic hemolysis: GWA interpretation

- IKZF1 (most significant SNP $p = 5.4 \times 10^{-8}$, near GW significant)
- ESYT2 (most significant SNP $p = 1 \times 10^{-7}$)
- G6PD and EPB41 (Erythrocyte Membrane Protein Band 4.1) and near significant. EPB41 is associated with Elliptocytosis-1
Manhattan Plot: GWA analysis Oxidative hemolysis in All Races. 4 genome wide significant hits
Oxidative hemolysis: GWA interpretation

• G6PD (most significant SNP p = 3E-18). This enzyme helps protect red blood cells from damage and premature destruction. Top SNP is rs1050828 (also referred to as G202A or **G6PD A-**) is associated with a reduction of G6PD. The G6PD deficient genotype A- causes an ~8-20% reduction of G6PD levels.

• GPX4 (Glutathione Peroxidase 4; most significant SNP p = 3.8E-14). The protein encoded by this gene belongs to the glutathione peroxidase family, members of which catalyze the reduction of hydrogen peroxide, organic hydroperoxides and lipid hydroperoxides, and thereby protect cells against oxidative damage.

• Chr 5 hit includes RHOBTB3, GLRX, and LINC01554. GLRX is Gluaredoxin. This gene encodes a member of the glutaredoxin family. The encoded protein is a cytoplasmic enzyme catalyzing the reversible reduction of glutathione-protein mixed disulfides. This enzyme highly contributes to the antioxidant defense system. Seems also to have a role in combating oxidative stress.

• Chr22 – SEC14L4 and SEC14L6. Not well characterized in humans. In yeast, the SEC14 protein is a phophatidylinositol transfer protein that is essential for biogenesis of Golgi-derived transport vesicles, and thus is required for the export of yeast secretory proteins from the Golgi complex.
The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe

Mehdi Nouraie,1 Janet S. Lee,2,3 Yingze Zhang,2 Tamir Kanas,2,3 Xuejun Zhao,4 Zeyu Xiong,2 Timothy B. Oriss,3 Qiliu Zeng,2 Gregory J. Kato,4 J. Simon R. Gibbs,5 Mariana E. Hildesheim,3 Vandana Sachdev,4 Robyn J. Barst,6 Roberto F. Machado,7 Kathryn L. Hassell,8 Jane A. Little,9 Dean E. Schaunfagel,7 Lakshmanan Krishnamurti,10 Enrico Novelli,2 Reda E. Girgis,11 Claudia R. Morris,12 Erika Berman Rosenzweig,6 David B. Badesch,8 Sophie Lanzkron,11 Oswaldo L. Castro,1 Jonathan C. Goldsmith,13 Victor R. Gordeuk,7* and Mark T. Gladwin,2,3* on behalf of the Walk-PHASST Investigators and Patients

1Howard University, Washington, USA; 2Vascular Medicine Institute, University of Pittsburgh, USA; 3Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, USA; 4Cardiovascular and Pulmonary Medicine Branch, NHLBI, Bethesda, MD, USA; 5National Heart & Lung Institute, Imperial College London, UK; 6Columbia University, New York, USA; 7University of Illinois, Chicago, IL, USA; 8University of Colorado HSC, Denver, CO, USA; 9Case Western Reserve University, Cleveland, OH, USA; 10Children’s Hospital of Pittsburgh, Pittsburgh, PA, USA; 11Johns Hopkins University, Baltimore, MD, USA; 12Children’s Hospital & Research Center Oakland, Oakland, CA, USA; and 13National Heart Lung and Blood Institute/NIH, Bethesda, MD, USA

ABSTRACT

The intensity of hemolytic anemia has been proposed as an independent risk factor for the development of certain clinical complications of sickle cell disease, such as pulmonary hypertension, hypoxemia and cutaneous leg ulceration. A composite variable derived from several individual markers of hemolysis could facilitate studies of the underlying mechanisms of hemolysis. In this study, we assessed the association of hemolysis with outcomes in sickle cell anemia. A hemolytic component was calculated by principal component analysis from reticulocyte count, serum lactate dehydrogenase, aspartate aminotransferase and total bilirubin concentrations in 415 hemoglobin SS patients. Association of this component with direct markers of hemolysis and clinical outcomes was assessed. As primary validation, both plasma red blood cell microparticles and cell-free hemoglobin concentration were higher in the highest hemolytic component quartile compared to the lowest quartile (P≤0.0001 for both analyses). The hemolytic component was lower with hydroxyurea therapy, higher hemoglobin F, and alpha-thalassemia (P≤0.0005); it was higher with higher systemic pulse pressure, lower oxygen saturation, and greater values for tricuspid regurgitation velocity, left ventricular diastolic dimension and left ventricular mass (all P<0.0001). Two-year follow-up analysis showed that a high hemolytic component was associated with an increased risk of death (hazard ratio, HR 3.44; 95% confidence interval, CI: 1.2-9.5; P=0.02). The hemolytic component reflects direct markers of intravascular hemolysis in patients with sickle cell disease and allows for adjusted analysis of associations between hemolytic severity and clinical outcomes. These results confirm associations between hemolytic rate and pulse pressure, oxygen saturation, increases in Doppler-estimated pulmonary systolic pressures and mortality (Clinicaltrials.gov identifier: NCT00492534).
Enrichment of association significance for *in vivo* hemolysis in SCD in osmotic SNPs identified in RBC-Omics Study

Hemolysis ~ SNP dosage + age + gender+ Hb genotype + HbF (hydroxyurea) + HbA (recent transfusion) + population structure
Potential Future Research Directions - Optimizing RBCs

- RBC precursors
  - Stem cells
  - Hematopoiesis

- Determinants of donor RBC survival
  - Gene/Environ/Omics

- Improved Additive Solutions

- Improved containers
  - ? Replace plasticizer Di-[2-Ethylhexyl]-Phthalate (DEHP)

- Optimizing the RBC products
  - Leukoreduction
  - Rejuvenation solutions
  - Washing
  - Freezing
  - Renitrosylation

- Better understanding RBC metabolism and its determinants

- Methods to measure RBC transfusion effectiveness such as tissue oxygenation

*Precision Transfusion Medicine*: RBC donor genotypes ascertained to inform time limits of blood storage, frequency of donation and recovery of cells after storage

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