Genetic, ethnic and sex determinants of red blood cell storage and stress hemolysis

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ABSTRACT

Donor genetic polymorphisms may affect red blood cell (RBC) structure, function and response to stress, which may significantly impact the survival of RBCs during cold storage and after transfusion. RBC donor genetic variability is anticipated to be high in donors with racial admixture from malaria endemic-regions, such as equatorial Africa, Asia, and South and Central America. The purpose of this study was to characterize the effect of donor’s race, sex, and age on RBC susceptibility to cold storage, osmotic or oxidative stress. We developed high throughput assays for these perturbations using RBC hemolysis as a read-out, and evaluated 11,223 consented donors from the Red Blood Cell-Omics study, enriched for African American, Asian and Hispanic donors. RBC concentrates (15 mL) from leukocyte-reduced units were stored in customized bags for 39-42 days (1-6°), and later evaluated for storage hemolysis, osmotic hemolysis (Pink test), and oxidative hemolysis using AAPH exposure. Male sex was strongly associated with increased susceptibility to hemolytic across all of our hemolytic assays. Donor’s age had sex-specific effects on storage and osmotic hemolysis in a mechanism that may involve sex hormones. RBCs from Caucasian donors hemolyzed less than the other racial groups after cold storage and in response to oxidative stress, whereas African American RBCs exhibited unique resistance to osmotic stress. These data exemplify the impact of donor genetic characteristics on the RBC storage stability, where further characterization of specific genetic modifiers of hemolysis may identify novel signaling pathways, predict RBC storage outcome, and improve donor eligibility for donation.
INTRODUCTION

Blood donors represent a genetically diverse population with inherited differences in red blood cell (RBC) characteristics that may modulate predisposition to hemolysis and RBC recovery in response to various stress conditions including cold storage of RBC units. While current blood-banking regulations have largely succeeded in reducing the incidence of donor-transmitted transfusion reactions, the effect of donor genetic and biologic factors such as race, sex or age on the RBC storage stability remains unclear. A growing number of studies have indicated the donor dichotomy in RBC characteristics\(^1\textendash}^4\) bringing up the distinction between donors whose RBCs exhibit poor storage recovery versus ‘super-donors’, whose RBCs are resilient to \textit{ex vivo} preservation. Although the clinical outcomes of such discrepancies have not been established in human, comparable studies in mice have demonstrated strain-specific susceptibility to RBC cold storage injury that was correlated with post-transfusion recovery.\(^5\)

Current understanding of genetic and biologic variables that may affect RBC storage quality is limited to known RBC disorders, where genetic enrichment for mutations related to hemolytic diseases, such as sickle cell disease\(^6\), thalassemia or glucose-6-phosphate dehydrogenase (G6PD)-deficiency, may modulate RBC rheology or antioxidant capacity, and may compromise RBC recovery during storage and after transfusion.\(^7\textendash}^8\) Genetic polymorphism that impacts RBC viability is anticipated to be high in donors with racial admixture from malaria endemic-regions, such as equatorial Africa, Asia, and South and Central America. With this regard, it has been suggested that there are as many as 1400 RBC mutations associated with malaria endemicity whose consequence on RBC characteristics has not been studied.

In addition to ethnic background, growing attention has been given to the effect of donor’s sex on predisposition to hemolysis and RBC storage outcome. Several studies have
indicated that male RBCs from human or mouse exhibit increased susceptibility to storage or stress-induced hemolysis\textsuperscript{2,9-11}, in a mechanism that is partially mediated by testosterone signaling.\textsuperscript{11} Conversely, a recent observational study has associated female sex and a young donor age with increased risk of post-transfusion mortality.\textsuperscript{12} These observations have inspired the National Heart, Lung and Blood Institute (NHLBI) and its Recipient Epidemiology Donor Evaluation Study (REDS)-III program to further characterize the donor differences in predisposition to hemolysis by establishing the Red Blood Cell-Omics (RBC-Omics) study.

RBC-Omics is a multi-center study conducted to identify genetic and biologic modifiers of hemolysis in blood donors that represent the donor population of the USA. We hypothesized that genetic variation in blood donors underlies the propensity of RBCs to hemolyze under cold storage or in response to selected perturbations including osmotic or oxidative stress. In the present study, we discuss the effect of donor’s racial background, sex or age on the RBC storage stability or response to applied stress conditions. We demonstrate race- and sex-specific differences in predisposition to hemolysis, for which male sex is strongly associated with increased susceptibility to storage and stress-induced hemolysis. We further demonstrate in both sexes that donor’s age is a significant modulator of storage and stress-induced hemolysis. Our findings elucidate the effect of donor characteristics on the quality of RBC products, and emphasize the requirement for better assessment of genetic and biological factors that may predict RBC storage outcome, and improve donor eligibility for donation.
MATERIALS AND METHODS

Humans Subjects: RBC-Omics was conducted under regulations applicable to all human subject research supported by federal agencies. The Domestic Coordinating Center (DCC) of REDS-III was responsible for the overall compliance of human subjects regulatory protocols including Institutional Review Board (IRB) approval from each participating blood center or the data coordination center (RTI International, Rockville, MD).

Study design and donor recruitment: Donor selection and recruitment for RBC-Omics was performed at four blood centers including American Red Cross (ARC, New Haven, CT), the Institute for Transfusion Medicine (ITxM, Pittsburgh, PA), Blood Center of Wisconsin (BCW, Milwaukee, WI), and Blood Centers of the Pacific (BCP, San Francisco, CA). Overall, 13,770 donors provided informed consent. Enrolled donors (n=13,538) were ≥18 years of age from five ethnic groups, which were self-reported by each donor. The ethnic groups included non-Hispanic Caucasian, Hispanic Caucasian, non-Hispanic African American, non-Hispanic Asian, and Other, which included mixed race, Hawaiian American, and Native American. In addition, we recruited a group of 1776 Caucasian “Super donors”. Selection criteria for these donors included 10 or more blood donations in prior 24-month without a low hemoglobin deferral for anemia.

Demographic data, including race and ethnicity for the enrolled donors were collected directly from the enrollment interview and recorded in the RBC-Omics Study Management System (SMS) database. Additional demographic data, including weight, height, BMI, and date of birth, as well as the donation history were pulled from donation centers’ donor donation databases and linked to the SMS database through Donor ID and donation date. The linking was further confirmed by the donation identification number (DIN). Donor age at enrollment
donation was derived by calculating the difference between enrollment date and donor date of birth and rounding down to year.

**Blood collection and components:** Whole blood units from eligible consented donors were processed according to each blood center operating procedures. Each whole blood unit was tested for complete blood count (CBC) and plasma ferritin levels (ng/dL) prior to the creation of leukocyte-reduced packed RBC (LR-pRBC) units in additive solution-1 or 3 (AS-1 or AS-3). A representative portion (10-15 mL) of RBCs from each LR-pRBC unit was then transferred into a FDA approved transfer bag (Haemonetics, Braintree, MA) using sterile docking. The LR-pRBC parent units were released for distribution as they were targeted for transfusion, whereas the transfer bags were sent to RBC-Omics testing labs (University of Pittsburgh, Pittsburgh, PA, and Blood Centers of the Pacific, San Francisco, CA) where they were stored under routine blood banking procedures (1-6° C) for 39-42 days.

**Evaluation of hemolytic propensity in stored RBCs:** Testing for spontaneous (end of storage) or stress-induced hemolysis was performed at RBC-Omics testing labs following storage in transfer bags as described above. Percent end of storage hemolysis was determined by comparing the supernatant hemoglobin (Hb\text{supernatant}) to total hemoglobin concentration (Hb\text{total}) of each stored RBC sample, and correcting for the hematocrit (HCT) level accordingly as described in the equation below. Hemoglobin micromolar concentrations were determined by the Drabkin’s method.\textsuperscript{13}

\[
\text{Storage hemolysis (\%)} = \frac{(100 - \text{HCT}) \times \text{Hb\text{supernatant}}}{\text{Hb\text{total}}}
\]

For the evaluation of stress-induced hemolysis, stored RBCs were washed (1500x g, 10 min, 18 °C) three times with phosphate-buffered saline (PBS) to remove additive solution. Washed RBC concentrates were then subjected to osmotic or oxidative stress as follows:
RBC osmotic fragility: osmotic fragility was determined by a modified pink test assay.14,15 Washed RBCs were incubated (4 h at 22°C) in pink test buffer (a hypotonic Bis-Tris buffer containing 25 mmol/L sodium chloride, 70 mmol/L Bis-Tris buffer, and 135 mmol/L glycerol; pH 6.6) at a final concentration of 1.6 % ± 0.2 % after which all samples were centrifuged (1500x g, 10 min, 18 °C), and percent osmotic hemolysis was determined by:

Osmotic hemolysis (%) = \frac{H_{\text{osmotic}}}{H_{\text{total}}} \times 100, \text{ where } H_{\text{osmotic}} \text{ corresponds to supernatant cell-free hemoglobin of pink test-treated RBCs.}

RBC oxidative hemolysis: Washed RBCs were suspended with PBS to a final concentration of 3.5 ± 0.5 % and later incubated (37°C) in the presence or absence of 2,2'-azobis-2-methylpropanimidamide, dihydrochloride (AAPH, 150 mmol/L) for 1.5 h after which all samples were centrifuged (1500x g, 10 min, 18 °C), and AAPH-induced oxidative hemolysis was determined by:

Oxidative hemolysis (%) = \frac{H_{\text{AAPH}} - H_{\text{control}}}{H_{\text{total}}} \times 100, \text{ where } H_{\text{AAPH}} \text{ corresponds to supernatant cell-free hemoglobin of AAPH-treated RBCs, } H_{\text{control}} \text{ corresponds to supernatant cell-free hemoglobin of untreated RBCs.}

Statistical analysis: Evaluation of the association between donor’s age and each hemolytic output reported in Figure 2 was performed by regression analysis using smoothing splines on donor ages. R package ggplot2 (version 2.1.0) and gam (generalized additive model, version 1.12), which uses a backfitting algorithm to combine different smoothing method (smoothing across 80 points). The fitted line indicates the predicted mean hemolysis for corresponding age, while the shaded areas highlights the 95% confidence interval for the standard error of the mean. Density plots for each of the hemolysis measures (Figure 3) were generated using the ggplot2 packages (version 2.1.0) in R statistical software for Windows, version 3.3.1. Distribution of storage hemolysis is strongly right skewed (skewness = 14.05), thus density plots were created
separately for donors with percent storage hemolysis measures between [0, 1] and those between [1, 13]. Correlation among the three hemolytic measures (Table 4), were assessed with the Pearson correlation coefficient. Statistical significance was determined using a t-test with N-2 degree of freedom, where N is the total number of donors with both hemolysis measures. Calculations were performed using R statistical software for Windows, version 3.3.1. The null hypothesis is reject when p-value is smaller than 0.01, to allow a modest correction for multiple tests.
RESULTS

*RBC-Omics donor demographics*: Between December 2013 and December 2015, we consented 13,770 blood donors out of whom 13,538 donors were enrolled in this study. RBCs from participating donors were stored for 39-42 days and later assessed for storage and stress-induced hemolysis as described in the Materials and Methods section and Figure 1. “Super donors”, who have donated 10 or more units 24 months prior to this study without a history of low hemoglobin deferral for anemia (n=1776), were excluded from our analyses to avoid bias caused by enrichment of primarily Caucasian donors who were enrolled based upon unique characteristics, and whose hemolytic scores may have been influenced by non-genetic factors, namely their large number of blood donations. To study the effect of donor’s predisposition to hemolysis based upon race, we determined the rate of storage and stress-induced hemolysis in donors from selected racial background (Caucasians, Hispanics, African-Americans, Asians, and Others) representative of the donor population in the US. Age-matched donors from both sexes were represented in this study (53.1 % females and 46.9 % males), whereas donors’ mean age varied among the five racial groups, for which the average age of Caucasian donors was higher (p<0.0001) than the rest of the groups. The demographic profile of the RBC-Omics donor cohort is summarized in Table 1.

*Male sex and aging in both sexes modulate RBC response to cold storage or hemolytic stress*: Male sex has been associated with increased susceptibility to hemolysis in stored RBCs from human and mice. Our evaluation of sex differences in predisposition to hemolysis confirms these observations by demonstrating significantly higher levels of hemolysis in male RBCs subjected to cold storage (39-42 days) or to stress-induced hemolysis, for which we have observed remarkable sex differences in RBC osmotic fragility (Table 2). The sex differences in
our hemolytic measurements were highly noticeable in younger donors (ages 18-45) of reproductive age, and to a lesser extent in older donors (Figure 2), suggesting sex hormone mediated effects.

Transfusion of blood units from younger donors (<30 years old) has been recently associated with increased risk of post transfusion mortality as compared with blood donated by older donors. Our evaluation of predisposition to hemolysis based upon donor’s age revealed age-specific differences in each of our hemolytic measurements (Figure 2). In the case of storage and osmotic hemolysis, aging affected male and female RBCs differently. In females, aging was associated with a steady increase in storage hemolysis levels (Figure 2A), whereas in males, the bell-shaped curve indicated that hemolysis levels sharply increase in RBC units donated by younger donors (ages 18-45), after which aging was associated with lower levels of storage hemolysis. This pattern was also observed when male RBCs were exposed to osmotic stress (Figure 2B). Conversely, younger age in female donors (18-45) was associated with enhanced resistance to osmotic hemolysis that was lost at middle age, but was observed again in elderly donors (>65 years old).

With regards to AAPH-induced oxidative hemolysis, donor’s age affected male and female similarly (Figure 2C). In both sexes, younger age (18-45) had a minor effect on predisposition oxidative hemolysis, whereas aging (>45 years old) was strongly associated with increased resistance to AAPH exposure. In comparison, average oxidative hemolysis in younger male and female donors (<21 years old) measured 38.9±9.3 % versus 33.6±9.7 % in older donors (>65 years old; p<0.0001 by variance t test).

Donor’s racial background is a significant modifier of predisposition to hemolysis in stored RBCs: Analysis of storage hemolysis by race revealed minor differences between RBCs
donated by Hispanic or African-American donors and Caucasian donors (Table 3 and Figure 3A). Conversely, RBCs donated by Asian donors exhibited significantly (p=1.45x10^-4) higher levels of hemolysis compared with Caucasians (0.40±0.36 % versus 0.36±0.21 %, respectively), whereas the lowest levels of storage hemolysis were observed in Other donors (mixed-race, Hawaiian-American, Native-American) (0.34±0.23 %). Further evaluation of race differences in storage hemolysis at levels exceeding FDA regulations (>1 %) (Figure 3A insert and Table 3; 95 percentile in storage hemolysis) suggested that the incidence of extreme hemolysis was more frequent in RBCs donated by African American or Asian donors than in Caucasians.

We further verified whether the race differences in storage hemolysis are intrinsic to the RBC using our stress hemolysis assays, which are performed on washed RBCs in the absence of additive solution or donor’s plasma. Evaluation of race differences in osmotic hemolysis reveled remarkable differences among the racial groups, for which Caucasian RBCs exhibited significantly higher levels of osmotic hemolysis compared with the other groups (Table 3 and Figure 3B). Most notably, RBCs from African-American donors exhibited an extraordinary resistance to osmotic lysis demonstrated by a left shift in the density distribution histogram (Figure 3B), for which the mean osmotic hemolysis (18.7±10.4 %) was significantly lower than the other groups (ranged from 25.8±12.7 % in Other donors to 30.6±12.7 % in Caucasians).

Donor racial background was also associated with predisposition to oxidative hemolysis (Table 3 and Figure 3C), for which Caucasian RBCs were more resilient to AAPH-induced hemolysis (36.7±9.6 %), compared with the other groups, whereas increased susceptibility to AAPH hemolysis was observed in RBCs donated by Hispanic donors (39.4±9.9 %).

Storage hemolysis is poorly associated with donor’s predisposition to osmotic or oxidative hemolysis: We anticipated that donor’s predisposition to storage hemolysis would
coincide with RBC susceptibility to stress-induced hemolysis (osmotic fragility and oxidative stress). We therefore determined the correlation (Pearson’s coefficient) between our selected hemolytic outputs (Table 4) and found a significant, but weak \((r=0.103)\) correlation between storage hemolysis and osmotic hemolysis, and no correlation \((r=0.019)\) between storage hemolysis and AAPH-induced oxidative hemolysis. Likewise, we found a weak correlation \((r=0.048)\) between our stress hemolysis assays. These data pointed out the uniqueness of each hemolytic stress condition that may be regulated by different genetic and molecular factors.
DISCUSSION

Our study provides new evidence regarding donor-specific differences in predisposition to hemolysis, for which donor’s sex, racial background or age, may significantly modulate the survival of RBCs following cold storage. Genetic mutations or biologic factors that modulate RBC responses to stress and favor hemolysis may increase the risk of transfusion reactions via enhanced destruction of stored RBCs in the patient circulation. Intravascular hemolysis releases cell-free hemoglobin, heme, arginase, ADP and other RBC metabolites that may induce hypertension and endothelial dysfunction by multiple mechanisms including hemoglobin mediated-nitric oxide (NO) scavenging\textsuperscript{16-18}, oxidative stress\textsuperscript{19} and inflammation.\textsuperscript{20} Such scenarios are particularly critical when transfusing patients with preexisting vascular dysfunction, a situation that may further enhance RBC or free hemoglobin interactions with the vasculature.

Several observational studies have recently investigated the association between donor characteristics and post-transfusion morbidity or mortality in certain patient populations. With regards to sex, two studies have associated female RBCs transfusion of with increased risk of death\textsuperscript{12,21}, one study found no association between donor’s sex and post transfusion mortality\textsuperscript{22}, whereas another study has indicated that male RBC transfusion may enhance the development of necrotizing enterocolitis in neonates undergoing transfusion.\textsuperscript{4,23} As packed RBC units contain plasma, these studies could only evaluate total blood component transfusion, for which donor’s plasma may have been responsible for transfusion-related injuries. Our evaluation of sex differences in storage and stress-induced hemolysis strongly supports the hypothesis that sex is a biologic factor that modulates predisposition to hemolysis in blood storage and across human disease.
Although the sex differences in storage hemolysis have been recently reported in a cohort of blood donors from Canada\textsuperscript{2,11}, a large-scale characterization of predisposition to stress-induced hemolysis has not been performed. Our observations of sex differences in washed stored RBCs subjected to osmotic or oxidative hemolysis (Figure 2B-C) suggest that the sex impact on predisposition to hemolysis is intrinsic to the RBC and is not mediated by factors related to donor’s plasma or additive solution. The molecular mechanisms that promote sex differences in hemolysis are not clear, however, a recent study has linked testosterone with enhanced susceptibility to hemolysis in male mice. In this study, orchiectomy improved the post transfusion recovery of stored RBCs as compared with male RBCs, whereas testosterone repletion in orchiectomy mice exacerbated RBC response to osmotic or AAPH-induced oxidative stress.\textsuperscript{11}

In support of a sex hormone-mediated mechanism, our analyses of sex differences in hemolysis by age revealed that although the sex difference is largely consistent across all ages (Figure 2), sex-specific patterns exist during donor’s reproductive years (ages 18-45) in storage (Figure 2A) and osmotic hemolysis, for which we have observed remarkable differences between the sexes (Figure 2B) with female RBCs becoming more resistant, and male RBCs more susceptible to osmotic fragility. Although the clinical relevance of these findings is yet to be established, comparable studies in mice have demonstrated that increased susceptibility to our stress hemolysis assays (pink test and AAPH), as observed in certain mouse strains or in male mice across strains, predicted poor post transfusion recovery of stored RBCs.\textsuperscript{11}

Interestingly, we found that aging in both sexes was accompanied with enhanced resistance to AAPH-induced oxidative hemolysis (Figure 2C). This finding was somewhat counterintuitive as oxidative stress is a hallmark of aging, and previous studies have
demonstrated increased levels of oxidative stress and compromised antioxidant activity in RBCs from elderly subjects (ages 50-75) as compared with younger subjects (ages 20-30).\textsuperscript{24} The mechanisms that confer resistance to AAPH oxidation in older blood donors are not clear, and may not be related to sex hormones as suggested by the similar trend observed in both sexes. One explanation may concern age-related changes in iron availability, for which reduced levels of iron stores in older donors may mitigate the rate of heme and iron-mediated oxidative stress. The recent report that linked transfusion of RBCs from older donors with reduced risk of post transfusion mortality\textsuperscript{12} may in part be explained by our observations; however, further characterization of age-related differences in RBC characteristics is required.

Our evaluation of race differences in predisposition to hemolysis further supports the hypothesis that donor genetic background underlies the propensity of RBCs to hemolyze during cold storage and under stress. We chose blood donors from four ethnic groups that best represent the donor demographics in the US, and compared the rates of hemolysis in each minority group to that of the majority non-Hispanic Caucasian donors (Figure 3 and Table 3). Among our three hemolytic assays, osmotic hemolysis has demonstrated the strongest effect of donor’s race on the rate of hemolysis demonstrated by distinct resistance of African-American RBCs to osmotic stress (Figure 3B). Conversely, RBCs from Caucasian donors exhibited lower levels of hemolysis after storage and in response to AAPH-induced oxidative stress (Table 3).

The clinical implications of the race differences in predisposition to hemolysis are not clear and it may be argued that the in the case of storage hemolysis (Figure 3A), the magnitude of the differences among the groups is relatively small. However, our evaluation of “extreme hemolyzers” (≥1 % storage hemolysis; Figure 3A insert and Table 3: storage hemolysis)
suggested that underlying differences may exist among the groups, which could ultimately affect the recovery of stored RBC \textit{in vivo}.

The unique resistance of African American RBCs to osmotic fragility may stem from the high prevalence of genetic traits to hemolytic diseases (sickle disease, thalassemia) in blood donors of African descent. A recent study, which evaluated the impact of sickle cell trait on the RBC storage stability and post transfusion recovery, has demonstrated that sickle trait RBCs exhibited high resistance to osmotic hemolysis along with accelerated degradation during cold storage, and reduced post transfusion recovery in mice.\textsuperscript{6} As sickle cell trait affects 8 to 10 \% of African Americans\textsuperscript{25}, other genetic factors may modulate the osmotic behavior of African American RBCs. In fact, racial differences have been reported in RBC cation transport including reduced membrane sodium pump activity in African Americans as compared with Caucasians.\textsuperscript{26,27}

Our observation of poor correlations among the hemolytic assays (Table 4) suggests that different genetic factors modulate predisposition to each stress condition. Another explanation is the impact of non-genetic factors (component processing, type of storage solution) that may modulate the rate of storage hemolysis. Since our study has focused on \textit{in vitro} correlates of hemolysis, the ability of each hemolytic output to predict RBC post transfusion recovery is unclear and further studies are required to determine which of these assays best predict transfusion outcomes.

In conclusion, our study offers new insights into the donor differences in predisposition to hemolysis, for which factors such as sex, age and racial background may significantly determine the survival of RBCs during storage and after transfusion. In addition, our hemolytic survey of 11,223 blood donors has identified individuals donors whose RBCs exhibited extreme
resistance (‘super donors’) or susceptibility to hemolysis. Such donors are invaluable for the subsequent discovery of genes and metabolic markers of hemolysis in RBC storage and in human disease.
REFERENCES


TABLES

Table 1: RBC-Omics donor demographics

<table>
<thead>
<tr>
<th></th>
<th>Females (n)</th>
<th>Males (n)</th>
<th>Females (age)</th>
<th>Males (age)</th>
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<tr>
<td>Caucasian</td>
<td>3411</td>
<td>2970</td>
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<td>611</td>
<td>403</td>
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<td>34.2±13.6</td>
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<td>African-American</td>
<td>880</td>
<td>731</td>
<td>40.1±15.8</td>
<td>39.1±15.5</td>
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<tr>
<td>Asian</td>
<td>737</td>
<td>869</td>
<td>34.9±13.4</td>
<td>36.7±12.</td>
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<tr>
<td>Other</td>
<td>326</td>
<td>285</td>
<td>35.3±14.5</td>
<td>37.2±14.3</td>
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Table 2: Sex differences in storage (39-42 days), osmotic or oxidative hemolysis (%). * designates significant (p<0.05 by t test) differences between the sexes.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>p value (t-test)</th>
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<th>Males (n)</th>
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<tr>
<td>Storage</td>
<td>0.34±0.28</td>
<td>0.40±0.28</td>
<td>*3.6x10^-22</td>
<td>5601</td>
<td>4983</td>
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<tr>
<td>Osmotic</td>
<td>25.9±12.3</td>
<td>30.4±13.6</td>
<td>*2.1x10^-69</td>
<td>5614</td>
<td>5010</td>
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<tr>
<td>Oxidative</td>
<td>36.6±9.9</td>
<td>38.5±10</td>
<td>*2.5x10^-18</td>
<td>4504</td>
<td>4029</td>
</tr>
</tbody>
</table>
Table 3: Effect of donor racial background on storage (top), osmotic (middle) or oxidative (bottom) hemolysis: p values were obtained by t tests, which compared the mean hemolysis value of each minority group with that of Caucasian donors. * p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>Storage hemolysis (%)</th>
<th>Osmotic hemolysis (%)</th>
<th>Oxidative hemolysis (%)</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>p value (t-test)</td>
<td>Range (Min-Max)</td>
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<tr>
<td>Caucasian</td>
<td>0.36±0.21</td>
<td>*4.59x10^-2</td>
<td>0.10-3.65</td>
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<tr>
<td>African-American</td>
<td>0.37±0.24</td>
<td>*1.45x10^-1</td>
<td>0.09-12.9</td>
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<tr>
<td>Asian</td>
<td>0.38±0.48</td>
<td>*0.03</td>
<td>0.11-3.03</td>
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<td>Hispanic</td>
<td>0.43±0.28</td>
<td>*0.03</td>
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<tr>
<td>Other</td>
<td>0.34±0.23</td>
<td>*0.03</td>
<td>0.11-3.03</td>
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Table 4: Correlation between hemolytic assays

<table>
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<th>Hemolysis</th>
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<tr>
<td>Storage v osmotic</td>
<td>0.103</td>
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<td>Storage v oxidative</td>
<td>0.019</td>
<td>0.09</td>
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<tr>
<td>Osmotic v oxidative</td>
<td>0.048</td>
<td>6.6x10^-6</td>
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</table>
FIGURE LEGENDS

Figure 1: Flowchart of the RBC-Omics study cohort and donor testing for hemolysis.

Figure 2: Effect of donor’s age and sex on RBC storage and stress-induced hemolysis. RBC concentrates from male or female donors ages 18-90 years old were stored (1-6° C) for 39-42 days and tested for A. percent storage hemolysis. B. Osmotic fragility (4 h Pink test). C. AAPH-induced oxidative hemolysis (150 mmol/L, 1.5 h, 37° C). The fitted line indicates the predicted mean hemolysis for corresponding age, while the shaded areas highlights the 95% confidence interval for the standard error of the mean.

Figure 3: Effect of donor’s racial backgrounds on RBC storage and stress-induced hemolysis. Density plots of percent hemolysis in stored (1-6° C; 39-42 days) RBC concentrates from donors of selected racial backgrounds* (non-Hispanic Caucasian, Hispanic Caucasian, non-Hispanic African American, and non-Hispanic Asian). A. Percent storage hemolysis at the range of 0 to 1 % (acceptable for transfusion) and above 1 % (A-insert). B. Osmotic fragility (4 h Pink test). C. AAPH-induced oxidative hemolysis (150 mmol/L, 1.5 h, 37° C). * For simplification, hemolysis data from ‘Other’ donors (mixed race, Hawaiian American, and Native American) is not presented in this figure, but data are available in Table 3.
FIGURES

Figure 1:

Donor recruitment

Consented (13,770)

Enrolled (13,938)
Not enrolled* (192)

African-American (1,649)
Asian (1,634)
Hispanic Caucasian (1,065)
Other / Multiracial (674)
Non-Hispanic Caucasian (6,823)
"Super Donors" / Caucasian (1,790)

Characterization of donor predisposition to hemolysis

Blood donation

RBC storage (59-42 days)
Plasma/DNA extraction

Testing and Analysis:
Subjects with 0 to 8 historical donations within two years (n=11,223)
Storage hemolysis (n=10,584)
Osmotic hemolysis (n=10,624)
Oxidative hemolysis (n=8533)
Figure 2A:

Storage Hemolysis by Age

Figure 2B:

Osmotic Hemolysis by Age
Figure 2C: Oxidative Hemolysis by Age

Figure 3A: Storage Hemolysis Density by Group
Figure 3A-insert:

Figure 3B:
Figure 3C:

Oxidative Hemolysis Density by Group

Density

0.00 0.01 0.02 0.03 0.04

0 20 40 60

Oxidative Hemolysis

Group
- AFRAMRCN
- ASIAN
- CAUCASIAN
- HISPANIC