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Blood, sweat, and tears: Red Blood Cell-Omics study objectives, design, and recruitment activities

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Abstract

BACKGROUND: The Red Blood Cell (RBC)-Omics study was initiated to build a large data set containing behavioral, genetic, and biochemical characteristics of blood donors with linkage to outcomes of the patients transfused with their donated RBCs.

STUDY DESIGN AND METHODS: The cohort was recruited from four US blood centers. Demographic and donation data were obtained from center records. A questionnaire to assess pica, restless leg syndrome, iron supplementation, hormone use, and menstrual and pregnancy history was completed at enrollment. Blood was obtained for a complete blood count, DNA, and ferritin testing. A leukocyte-reduced RBC sample was transferred to a custom storage bag for hemolysis testing at Storage Days 39 to 42. A subset was recalled to evaluate the kinetics and stability of hemolysis measures.

RESULTS: A total of 13,403 racially/ethnically diverse (12% African American, 12% Asian, 8% Hispanic, 64% white, and 5% multiracial/other) donors of both sexes were enrolled and ranged from 18 to 90 years of age; 15% were high-intensity donors (nine or more donations in the prior 24 mo without low hemoglobin deferral). Data elements are available for 97% to 99% of the cohort.

[†]A complete list of the REDS-III RBC-Omics Study group members is located in Appendix S3.

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CONFLICT OF INTEREST

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CONCLUSIONS: The cohort provides demographic, behavioral, biochemical, and genetic data for a broad range of blood donor studies related to iron metabolism, adverse consequences of iron deficiency, and differential hemolysis (including oxidative and osmotic stress perturbations) during RBC storage. Linkage to recipient outcomes may permit analysis of how donor characteristics affect transfusion efficacy. Repository DNA, plasma, and RBC samples should expand the usefulness of the current data set.

Wide variation exists in donor iron metabolism, hemoglobin production, and end-of-storage stress hemolysis.[1] Additional genomic variability in erythrocyte protein expression and function have evolved in response to endemic malaria, suggesting potential donor heterogeneity in RBC structure and function during cold storage and in posttransfusion erythrocyte recovery.[2,3] Despite this variation, current blood banking guidelines regulating blood donation frequency, donation volume, and storage time and conditions remain uniform for all donors. The Red Blood Cell (RBC)-Omics study was conducted by the Recipient Epidemiology and Donor Evaluation Study (REDS)-III program, initiated by the National Heart, Lung, and Blood Institute (NHLBI) to include studies related to the quality of blood components and to examine molecular pathways relevant to transfusion medicine. Specifically, the RBC-Omics study had these aims:

1. Establish a multiethnic cohort of blood donors with well-characterized demographic, behavioral, and donation history.
2. Develop a database linking donations from these blood donors to outcomes in transfusion recipients; this linked database is a subset of a much larger linked donor-recipient data set.[4]
3. Identify genetic factors impacting hemoglobin, ferritin, and iron metabolism.
4. Identify genetic factors impacting maintenance of hemoglobin and iron stores in high-intensity blood donors.
5. Identify polymorphism(s) associated with the iron-related disorders pica and restless leg syndrome (RLS).[5–7]
6. Define the genetic and metabolic basis for donor-specific differences in spontaneous, osmotic, and oxidative hemolysis at the end of storage.
7. Establish a sharable biorepository of RBCs, plasma, white blood cells (WBCs), and DNA from the enrolled blood donors.

This article describes the design, implementation, and recruitment into the RBC-Omics study.

MATERIALS AND METHODS

Overview of screening phase

The RBC-Omics cohort was recruited from four large blood centers across the United States: the American Red Cross (ARC, Farmington, CT), the Institute for Transfusion Medicine (ITxM, Pittsburgh, PA), BloodCenter of Wisconsin (BCW, Milwaukee, WI), and the Blood Centers of the Pacific (BCP, San Francisco, CA). Leukoreduced (LR) RBC units

were collected from consenting donors and processed by component lab staff at each blood center to produce a 15-mL LR-RBC sample that was shipped to either the University of Pittsburgh Medical Center (UPMC, if collected at ITxM, ARC, or BCW) or the Blood Systems Research Institute (BSRI) (if collected at BCP) for hemolysis assays. LR filters were labeled and retrieved for the study, from which WBCs were eluted, aliquoted, and stored frozen for DNA isolation and genetic studies. Fieldwork in each location was implemented by trained blood center staff in coordination with a centralized Data Coordinating Center (DCC, RTI International, Rockville, MD) under the guidance of the REDS-III program Steering Committee and NHLBI program staff (Fig. 1).

Inclusion criteria

Participants included men and women who were 18 years and older and successfully donated whole blood at one of the four REDS-III blood centers. Self-reported race/ethnicity (African American, Asian, white, Hispanic, and Other [Native American, nonwhite Hispanic, and multiracial]) was used to track enrollment. Enrollment strategies varied by center and were designed to be integrated into routine blood center procedures through the training of key blood center registration, collections, and laboratory staff. All blood centers pursued targeted enrollment strategies to increase recruitment of minority (African American and Asian) and white so-called high-intensity donors (those who had provided nine or more RBC-equivalent donations (double RBCs qualify as two) in the preceding 24-month period, excluding the enrollment donation, without a low hemoglobin deferral. Enrollment began in December 2013 and was conducted over a 22-month period ending in October 2015.

Consent for study enrollment

Consent materials presented to respondents were standardized across the different centers. All recruitment materials and protocols were approved by each blood center's institutional review board (IRB) as well as by the DCC IRB.

Enrollment visit activities

Written informed consent was obtained from all participants before enrollment. Self-reported race/ethnicity was collected by study staff and recorded in the Study Management System (SMS) database during the enrollment interview. Donation date, donation identification number (DIN), and demographic data, such as weight, height, date of birth, education, and country of birth, along with prior donation history, deferral, and smoking history, were subsequently retrieved from blood centers' donor/donation databases or from supplementary donation questions unique to REDS-III. An RBC-Omics questionnaire, adapted from the REDS-II Donor Iron Status Evaluation (RISE) study,[8,9] was used to gather information on the use of medications that could affect iron status or RBC storage characteristics including use of iron supplements, antacids, and hormonal therapies, as well as menstrual and reproductive history for female subjects. Questionnaire content for pica and RLS were developed and administered to all enrollees (Appendix S1). RLS questions were taken from the validated Cambridge-Hopkins RLS Symptoms Questionnaire.[10,11] Both electronic and paper versions of the questionnaires were made available to participating hubs.

Before study initiation, the ability of the full study questionnaire to assess pica, RLS, medication use, and menstrual and pregnancy history was tested on nine blood donors via cognitive interviews conducted by a trained survey methodologist. The cognitive interview process included administration of the questionnaire along with the use of verbal probes to determine if the questions were clear, understandable, and matched the participant's experience of symptoms following blood donation. Blood donor feedback from the pretest was subsequently used to improve the comprehensibility of questions and to enhance the validity and reliability of the questionnaire. Questions about diet were not included because previous studies among donors revealed that dietary differences do not significantly alter iron status in a manner that can be reliably detected by a self-reported dietary history.[12,13]

During the enrollment visit, a whole blood unit and ethylenediaminetetraacetic acid (EDTA) retention tube were collected from each participant. The whole blood donor units were processed into LR-RBC components according to each blood center's standard operating procedures. Using routine sterile docking procedures, a 10-to 15-mL sample of LR-RBC in additive solution was transferred into a study transfer bag (Appendix S2), made specifically for this study by Haemonetics Corporation using the same materials as the parent RBC storage bag. All centers used the same transfer bag. The EDTA retention tubes, RBC LR filters, and transfer bags containing LR-RBC were maintained by the study; the LR-RBC parent units were released into blood center inventories for issue to hospitals for transfusion. All specimens derived from donations with a reactive infectious disease test result were discarded, and the corresponding donors were notified and unenrolled.

A complete blood count (CBC), including the collection of key RBC indices, was performed using the EDTA retention tube specimen. The Sysmex CBC machine was used to process EDTA specimens at three of the four blood centers; ITxM utilized the Beckman Coulter analyzer. Plasma was collected from remaining EDTA retention tube blood and frozen for batched ferritin testing, and two 0.5-mL aliquots of parent RBC (pRBC) and two 0.5-mL aliquots of plasma were frozen within 24 to 48 hours of collection for inclusion in the biorepository. All ferritin concentrations were obtained by batch testing using a quantitative latex agglutination assay on a chemistry system (AU680, Beckman Coulter). LR filters were processed to recover WBCs by back elution of cells using 40 mL phosphate-buffered saline (PBS). The WBC suspensions were aliquoted and frozen in four 1.8-mL cryovials, three for inclusion in the biorepository and one for DNA extraction and for genome-wide association study (GWAS) analysis. In total, 10 aliquots derived from each enrollment visit were prepared and used for a planned analysis or frozen and stored in the study repository (Supplementary Table S1).

Consenting participants were not considered fully enrolled in the study until a whole blood unit was successfully collected; double RBC donors and deferred donors were not eligible. A variety of other circumstances (e.g., quantity not sufficient [QNS] collections, blood clotting, filter failures, reactive test results, and broken bags) also resulted in the donor not being considered fully enrolled. Donors with these exceptions, excluding those with reactive test results, were eligible for reenrollment at a subsequent whole blood donation.

Targeted recruitment process

By design, the study oversampled Asian, African American, and Hispanic donors to support analysis of genetic differences across race/ethnicity. Recruitment was also enriched for high-intensity donors to allow for analysis of genetic polymorphisms associated with phenotypes relating to iron absorption/metabolism and symptoms of iron deficiency. Excluding the high-intensity cohort, however, exclusion criteria were few with study entry conditional on the following: signed informed consent, completion of study questionnaire, successful whole blood donation, and willingness to consider a recall donation. Given the limited nature of requirements for study entry, there is little a priori reason to suspect important differences between those enrolling in the African American, Asian, Hispanic, and white (non–high intensity) cohorts and their underlying populations. Consequently, this racially and ethnically diverse population of blood donors with a broad range of recent donation intensity is likely to be broadly representative of the REDS-III blood center populations.

Selection criteria for donors enrolled into the recall arm of the study

A selected set of donors with extreme high or low values on at least one of three hemolysis measures (top or bottom fifth percentile of each measure, adjusted for race and center) were identified and recalled for a second donation visit. These recall visits occurred between June 2014 and May 2015 and consisted of an additional informed consent followed by collection of a unit of whole blood and an EDTA retention tube that were processed (including sterile docking of a 10-to 15-mL sample of LR-RBC in an additive solution into a study transfer bag) and tested for CBC and ferritin. The recall LR-RBC units were used to examine kinetics of hemolysis at multiple time points during storage, as well as longitudinal reproducibility of end-of-storage spontaneous and stress hemolysis (osmotic, oxidative) measures across each of their two donations.[1,14] To allow for this comparison, hemolysis assays were performed after 39 to 42 days of storage on both samples from the parent LR-RBC units and transfer bag derived LR-RBC shortly after collection.

Recall visit components

Following the recall visit, the EDTA retention tubes, LR filters, transfer bags containing representative LR-RBC, and LR-RBC parent units were all maintained by the study. Laboratories processed the retention tube specimen into two 0.5-mL aliquots of plasma for frozen storage and inclusion in the biorepository, and one 2.5-mL aliquot of EDTA whole blood was transferred to a Tempus tube for RNA preservation that was frozen to allow for potential transcriptomic analyses (not a funded activity in RBC-Omics). The LR filters were processed to recover WBCs by back elution of cells from the filters using 40 mL PBS. The WBC suspensions were then aliquoted into five 1.8-mL vials for frozen storage and potential future inclusion in the biorepository. Ten whole blood aliquots were prepared and used for a planned analysis or frozen and stored during the recall visit. Additional detail on specimens collected during the enrollment and recall phases can be found in Supplementary Table S1.

Data management and transfer

The final RBC-Omics data set was constructed from multiple data sources, as shown in Fig. 2. All data collection and transfer was managed and monitored by the DCC. Using two-

factor authentication, blood center and laboratory staff entered data into secured data systems with direct submission to the DCC for periodic data merging. The REDS-III DCC maintained and accessed a donor-donation database that was used to obtain donation history for the 24 months preceding enrollment. Data from enrollment and recall visits were captured by the RBC-Omics SMS housed within a web-based REDS-III Domestic SMS framework hosted and managed by the DCC. Enrollment questionnaire responses were captured by a web-based form hosted within the REDS-III website, and data were immediately transferred to the DCC Enhanced Security Network, a Federal Information Processing Standards moderate environment. All biological and sample tracking information was entered directly into the Biological Specimen Inventory (BSI) system. Remaining data files were either shipped on encrypted media or uploaded by center staff to the DCC using the secure upload module within the REDS-III website (Fig. 2).

Quality assurance and control

A unique subject ID was assigned to each donor at the time of obtaining consent. The subject ID was used by the DCC within the RBC-Omics SMS to track participants through enrollment and completion of the study visit, the questionnaire, and laboratory testing. It served as a unique identification link between records in the SMS, the source records at each blood center, blood samples tracked in BSI, test results, and other relevant study data. Sample IDs were also assigned and provided an identification link for biospecimens collected from each specific subject. The DCC monitored the study remotely via centralized monitoring. This included monitoring blood center enrollment and adherence to the study timeline, reviewing reports generated by the electronic data capture system, programming edit checks, and monitoring the timeliness of data entry and queries and their resolution. Checks were routinely performed to identify potential anomalies such as missing, out-of-range, inconsistent, and illogical data. Center staff were queried by the DCC study manager via e-mail, and anomalies were typically resolved within 10 business days. The DCC study manager also convened regular conference calls so that sites could collectively discuss accrual challenges and plans for mitigation. For enhanced data quality assurance, data fields existing in more than one database were cross-compared among the independently maintained databases. Storage, osmotic, and oxidative hemolysis, ferritin, and CBC measures were reviewed for accuracy within normal ranges and linked to the SMS and BSI databases. Values were compared with reference ranges for the assays and typical distributions for variables and potentially swapped data such as height for weight. Potential discrepancies were assessed by querying the blood centers to verify values. Corrections were then populated back into the databases. Values below the limits of detection (LOD) for ferritin were replaced with the LOD of the assay plus a random normal value with mean 0 and the same standard deviation (SD = 0.5) as the ferritin measures above the LOD.

Spontaneous and stress-induced hemolysis testing

High-throughput assays for the evaluation of genetic and behavioral (e.g., donation frequency, iron/hormone therapies) factors in blood donors that may impact RBC function and survival in cold storage and after transfusion, as detailed by Kanias and colleagues,[1] were developed, validated, and performed at the UPMC (if collected at ITxM, Yale-ARC, or BCW) or BSRI (if collected at BCP). Two 1-mL aliquots used to quantify spontaneous

storage and stress-induced hemolysis were obtained from the transfer bag stored for 39 to 42 days at 1 to 6°C; these transfer bags had been prepared from each LR-RBC at the time of component manufacturing.

Spontaneous storage hemolysis has been used as the prime endpoint in quality assessment of RBC units. Stress hemolysis assays tested for donor variation in RBC osmotic fragility (pink test), mechanical fragility (recall phase only), and predisposition to oxidative hemolysis using an oxidizing agent that promotes membrane lipid peroxidation. Although these in vitro assays impose nonphysiologic perturbations, they provide reliable measurements of donor-specific differences in RBC function and membrane integrity in mice[3] and may predict genetic or sex differences in posttransfusion recovery of stored RBCs.

During the recall phase, a subset of donors identified as having an extreme hemolysis phenotype for one of three hemolysis measures was enrolled. The transfer bags derived from LR-RBC components manufactured from the recall visit donation were processed using the same procedures used during the enrollment phase and were tested at the end of the storage period (39–42 days after collection) for spontaneous storage and stress-induced hemolysis; these included the same assays as in the enrollment phase (e.g., spontaneous, osmotic, and oxidative hemolysis assays) along with the addition of a mechanical hemolysis assay. In addition, the full LR-RBC unit was accessed by sterile docking at two additional storage time points (8–12 and 18–23 days) for this expanded panel of hemolysis tests.

Whole genome genotyping

A customized Affymetrix Axiom Array, the Transfusion Medicine (TM) Array, was designed and used to genotype the cohort. This array includes approximately 879,000 single nucleotide polymorphisms (SNPs). The array includes SNPs to provide good imputation coverage (approximately 95% of markers had $R^2 > 0.8$ down to a minor allele frequency of 5%) in populations of European, African, and East Asian descent. Further details are in our companion article, “Development and Evaluation of a Transfusion Medicine Genome Wide Genotyping Array.”[13]

Metabolomics

To identify metabolites that correlate with RBC storage characteristics, LR-RBC samples from 200 recalled donors with values in the top or bottom 20th percentile for at least one hemolysis phenotype were collected at three storage time points (around Day 10, Day 21, and Day 42). The LR-pRBC aliquots were rapidly frozen in liquid nitrogen and compiled and used by Bloodworks Northwest and the University of Colorado to perform targeted and untargeted ultra-high-pressure metabolomic analyses based on mass spectrometry.[15] The data, which are in the process of being generated at the time of preparation of the current manuscript, will be stored, creating an electronic mass spectrometry data repository to investigate specific metabolite or metabolic patterns occurring during blood storage in the context of storage hemolysis phenotypes and GWAS findings. The data set will provide a unique opportunity to correlate metabolic phenotypes (metabotypes) to donor demographics, fresh and stored blood parameters, and genetic findings generated in RBC-Omics (e.g., age, sex, ethnicity, CBC values, ferritin levels, spontaneous and stress hemolysis levels during

storage, GWAS SNPs), thereby potentially generating information relevant to the fields of transfusion medicine and hematology at large.[16–18]

RESULTS

Of the 14,520 consenting donors, 13,770 (95%) were enrolled after successfully donating a whole blood unit. The 750 consented donors who did not complete the enrollment donation were not enrolled in the study. Reasons included deferral, incomplete donations (e.g., QNS), or donation of plasma or platelets rather than whole blood. After careful review, a total of 367 additional donors were excluded from the study after the enrollment visit because they 1) had informed consent issues; 2) enrolled a second time; 3) failed to obtain informative biological samples required for one or more of the planned analyses: ferritin, CBC, hemolysis, genotyping; 4) were incorrectly coded as giving a full unit when they did not; or 5) tested reactive for infectious disease markers. After these exclusions, 13,403 donors were considered fully enrolled in and informative for the study. Ferritin was measured at enrollment for 13,323 donors (99%); CBC results were available for 13,062 donors (97%), and GWAS TM Array data were available for 13,125 donors (98%). Of these 13,403 donors, a total of 664 were successfully recruited into the recall study (where a separate consent was obtained and the entire LR-RBC unit acquired for the study) (Fig. 3).

Enrollment was comparable across all four blood centers (Table 1). Of the 13,403 donors, 3355 were enrolled at BCW, 3292 were enrolled at ARC, 3192 were enrolled at ITxM, and 3564 were enrolled at BCP. Given the wide geographic spread, it was anticipated that each blood center would have a different distribution of racial/ethnic and high-intensity donation groups. As expected, based on previous blood center donation history, most high-intensity donors (46%) and non-Hispanic Asian donors (67%) were enrolled at BCW and BCP, respectively; ARC accounted for 54% of Hispanic donor enrollment (Table 1).

Table 2 describes the participant demographics. Demographic data and donation history were available for all 13,403 enrolled participants. Data on medication usage and menstrual and pregnancy history were available for 13,300 (99%) participants, as were pica and RLS questionnaire data. The cohort was evenly divided between female and male donors (6737 and 6666, respectively). There was a wide spread in age, with participants ranging from 18 to 90 years; most (61%) were 40 years or older. Most (70%) donors had donated in the previous 24 months; approximately 25% had donated seven or more times in the past 24 months, but most (53%) had donated two or fewer times. Most participants (76%) were born in the United States, did not smoke (80%), and did not take iron supplements (62%). Approximately 60% of all female participants had ever been pregnant including 74% of female high-intensity donors, 63% of white donors, 62% of African American donors, 49% of Hispanic donors, and 38% of Asian donors.

Biological specimen and data repository information coordinating center and database of genotypes and phenotypes

After the conclusion of the REDS-III program, which is scheduled to end in March 2019, RBC-Omics data will be made available for public access. The clinical data and procedures for access to repository samples will be deposited in the Biological Specimen and Data

Repository Information Coordinating Center (BioLINCC) (https://biolincc.nhlbi.nih.gov/home/). Genomic data will be deposited in the database of genotypes and phenotypes (dbGAP) (https://www.ncbi.nlm.nih.gov/gap). The metabolomics data will be deposited in the Metabolomics WorkBench (http://www.metabolomicsworkbench.org/). All the data files will include the same IDs, so the data across repositories can be linked for analyses.

Linked donor-recipient database

The REDS-III domestic program established a large research database[4] that links data from blood donors and their donations, the components made from these donations, the recipients of these components, and the clinical efficacy and adverse complications following transfusions. By retaining donor linkage, RBC-Omics will be able to 1) evaluate associations between clinical outcomes and transfusions of RBC with defined RBC storage and genetic characteristics, and 2) determine whether these genetic and metabolomic characteristics translate into demonstrable differences in hemoglobin increments and other recipient outcomes following transfusion of RBC components from RBC-Omics donors.

DISCUSSION

The REDS-III RBC-Omics study provides a unique resource for transfusion medicine research studies. The marked racial/ethnic diversity, size of the cohort, and depth of data allows for comparisons of interactions between race/ethnicity and several variables including differential RBC storage capacity, iron-related conditions associated with blood donation (e.g., iron deficiency, pica, and RLS), and other donation characteristics provided through the collection of questionnaire data. RBC-Omics participants also have TM Array GWAS data, and a minority of donors (who were recalled based on the hemolysis values of their enrollment unit) will also have metabolomic data generated over the course of RBC storage. These data will be made publicly available through dbGAP, Metabolomics Workbench, and BioLINCC to allow additional studies.

It is known that wide genetic variation exists among donors in iron metabolism and hemoglobin production.[8,12,13] For example, some blood donors can donate repeatedly without hemoglobin deferral, yet others are deferred for low hemoglobin after one or a few donations. Investigating whether there are DNA polymorphism(s) that enable high-intensity donation without low hemoglobin deferral or are associated with pica and RLS will contribute to understanding the physiologic mechanisms of iron absorption and metabolism. The questions used to assess the presence of RLS were well validated,[10,11] but those used to assess pica were developed for this study using cognitive interviews of nine individuals, five of whom experienced pica symptoms. Despite this effort, continued refinement of questions is needed to accurately detect pica symptoms by survey of blood donors.[19] A better understanding of these mechanisms may also enhance the overall donation experience by identifying new strategies to improve donor safety and reduce adverse side effects of blood donation.

Genetic and metabolomic data will address factors that influence the “RBC storage lesion.”[2] These results could potentially motivate a change in current blood banking practices[9,20,21] including donor eligibility criteria, donation frequency, and RBC component storage

policies. Further, results from RBC-Omics may lead to the development of new blood donor biomarkers that predict improved storage and enhanced posttransfusion RBC recovery. The findings may inform approaches for stabilizing RBCs during storage, such as improved storage solutions and containers.

The REDS-III program database can trace components donated by RBC-Omics donors over a 4.5-year period (July 2012-July 2016) that includes intervals preceding and following their RBC-Omics enrollment donation. Thus RBC-Omics may be able to evaluate associations between clinical outcomes of transfusion of RBCs with defined RBC storage and genetic characteristics to assess whether certain genetic and metabolomic characteristics translate into demonstrable differences in recipient outcomes. Because each individual blood center retains linkage to donor-identifying information, selected recall of donors with particular phenotypes/genotypes is possible and could be pursued in future studies including RBC functional experiments and autologous RBC recovery and survival studies to validate the relevance of identified genetic variants. Finally, the large RBC-Omics biospecimen repository will offer the potential for other investigators to develop personalized or precision medicine approaches to blood banking and RBC transfusion.

As with most studies, the RBC-Omics study was confronted with many successes and challenges. The recruitment was so successful due to the targeting of blood donors who are already inclined to volunteer and be willing to provide blood samples, often a barrier in many studies, and also understand the importance of biomedical research. We had lower than expected minority recruitment at some sites and higher than expected at others. Resources were identified to allow more successful sites to recruit more minority blood donors. At the end of the study, several sites also started losing research nurses as they were hired into new studies. White donor recruitment was ahead of anticipated and consequently was halted early to allow staff to focus on minority recruitment. Due to central lab holidays, we occasionally had to halt recruitment temporarily so that samples would not be wasted with no staff available to run hemolysis assays.

The RBC-Omics is a unique resource compared with other large blood donor studies. For instance, although the SCANDAT study covered the entire countries of Sweden and Denmark and has produced numerous important observations on donor health outcomes and impact of transfusions relative to donor characteristics,[22–25] it relied heavily on electronic medical record data, and little information could be collected directly from the donors.[26] Instead, SCANDAT is based on linkage of multiple extant registries in Sweden and Denmark using national identification numbers assigned to all citizens at birth. These include blood donation and transfusion information from these countries' national blood bank databases; detailed hospital and outpatient electronic medical records; birth, pregnancy, disease, and death registries; and socioeconomic data. SCANDAT does not obtain specific informed consent or obtain specimens from donors and hence has not conducted laboratory studies of genetic or other biological factors in donors or blood components.

The Danish Donor Cohort recently added specific consent for collection, retention, and testing of DNA and plasma samples from a subset of blood donors in Denmark.[27] This study is in the process of generating GWAS and other laboratory data to allow analyses of donor

genetic and biological factors that may impact downstream donor health and transfusion efficacy and complications. However, they have not acquired and stored samples of RBCs for evaluation of storage hemolysis parameters. Moreover, unlike RBC-Omics that tried to enroll (and indeed enrich for) ethnic minority donors, both the SCANDAT and Danish Donor Studies are composed of relatively homogeneous Scandinavian populations.

The Interval study, recently conducted by the National Health Service Blood and Transplant in the United Kingdom, randomized 45,000 donors stratified by gender to consent to specified donation frequency intervals to determine the impact of donation frequency on cumulative donation success and donor health outcomes. The Interval study[28,29] is a randomized clinical trial of primary white (>91%) blood donors. RBC-Omics, in contrast, is racially and ethnically heterogeneous. Interval includes a GWAS[30] using the UK BioBank Array and other laboratory assays including ferritin testing but did not perform RBC storage hemolysis assays. A working group of scientists from each of these studies has been established to conduct collaborative analyses where appropriate, and to inform the design and execution of future studies to advance the concept of precision transfusion medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

ARC	American Red Cross
BCP	Blood Centers of the Pacific
BCW	BloodCenter of Wisconsin
BioLINCC	Biological Specimen and Data Repository Information Coordinating Center
BSI	Biological Specimen Inventory
BSRI	Blood Systems Research Institute
dbGAP	database of genotypes and phenotypes
DCC	Data Coordinating Center
DIN	donor identification number
GWAS	genome-wide association study

ITxM	Institute for Transfusion Medicine
LOD	limits of detection
LR-RBC	leukoreduced red blood cell
NHLBI	National Heart, Lung, and Blood Institute
QNS	quantity not sufficient
RLS	restless leg syndrome
SMS	study management system
SNPs	single nucleotide polymorphisms

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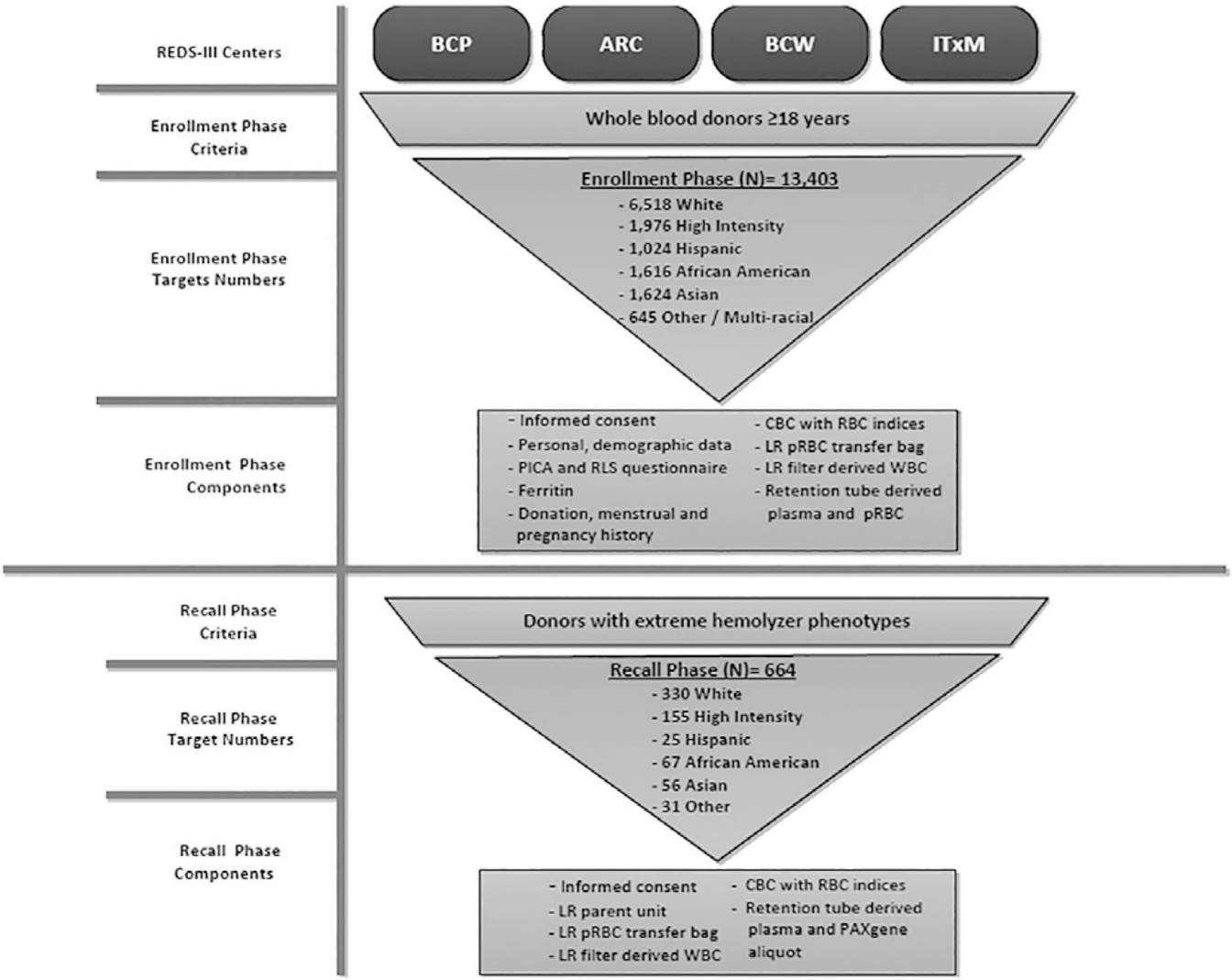

Fig. 1.
RBC-Omics study cohort.

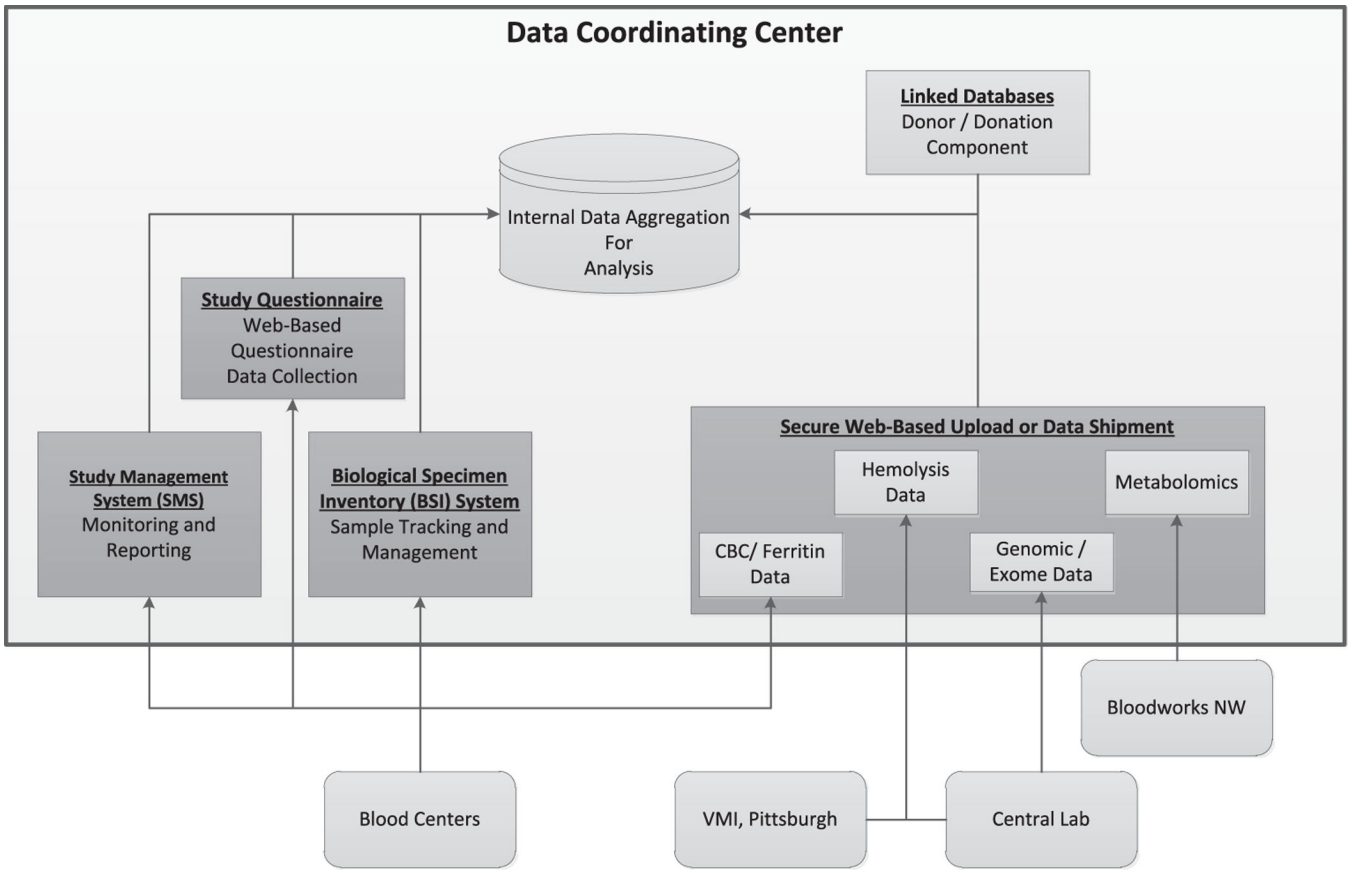

Fig. 2.
RBC-Omics study data management.

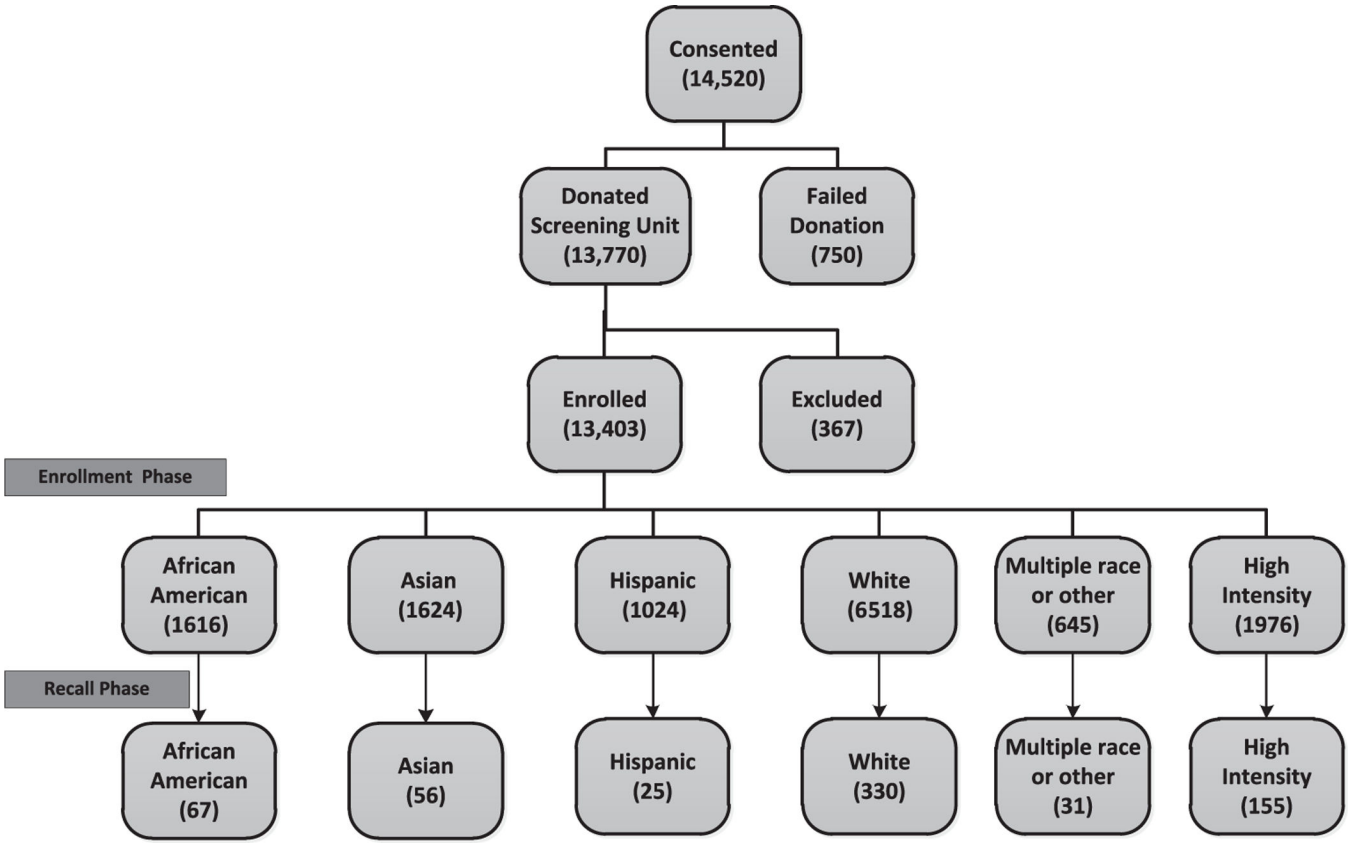

Fig. 3.
Flowchart of RBC-Omics study cohort.

TABLE 1.

Screening phase enrollment by blood center

Screening enrollment by blood center												
	White		African American		Hispanic		Asian		High intensity		Multiple race or other	
Blood center	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
BCW	1754	27	348	22	44	4	59	4	912	46	238	37
ARC	1081	17	598	37	551	54	363	22	573	29	126	20
ITxM	2103	32	453	28	44	4	114	7	449	23	29	4
BCP	1580	24	217	13	385	38	1088	67	42	2	252	39
Total	**6518**	**49**	**1616**	**12**	**1024**	**8**	**1624**	**12**	**1976**	**15**	**645**	**5**

TABLE 2.

RBC-Omics study participant demographics by racial ethnic group

Characteristic	White		African American		Hispanic		Asian		High intensity		Multiple race or other	
Total enrolled, screening phase	6518		1616		1024		1624		1976		645	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
N	3001	3517	746	870	409	615	884	740	1321	655	305	340
Blood center												
BCW	652	1102	156	192	11	33	24	35	545	367	110	128
ARC	647	434	291	307	236	315	220	143	430	143	58	68
ITxM	946	1157	176	277	26	18	55	59	308	141	17	12
BCP	756	824	123	94	136	249	585	503	38	4	120	132
Age, y												
18–20	77	134	70	83	52	90	50	74	0	0	24	28
20–29	467	568	188	204	135	202	245	248	32	11	77	129
30–39	432	482	110	154	83	132	244	177	68	30	69	56
40–49	464	561	134	151	68	100	172	114	140	62	56	48
50–59	777	916	152	157	51	68	120	83	404	235	46	52
60+	784	856	92	121	20	23	53	44	677	317	33	27
Donation frequency, past 24 mo												
0	751	923	280	399	179	298	456	406	0	0	134	172
1–2	732	900	244	300	113	196	230	193	0	0	77	91
3–4	530	667	113	97	59	79	82	80	0	0	33	30
5–6	481	510	50	43	26	24	62	29	0	0	17	18
7+	507	517	59	31	32	18	54	32	1321	655	44	29
Weight, mean SD	197.5 ± 37.5	165.3 ± 36.9	209.7 ± 48.7	180.2 ± 40.7	193.7 ± 37.0	164.6 ± 38.2	172.8 ± 28.3	142.2 ± 25.1	201.7 ± 37.1	163.6 ± 33.5	189.2 ± 36.9	165.2 ± 40.1
Height, mean SD	70.5 ± 2.9	65.1 ± 2.7	70.2 ± 3.2	64.9 ± 2.8	68.9 ± 3.0	63.7 ± 2.6	68.1 ± 2.7	63.4 ± 2.3	70.2 ± 3.0	64.6 ± 2.6	69.7 ± 3.2	64.9 ± 2.9
BMI, mean SD	27.9 ± 5.0	27.5 ± 6.1	29.9 ± 6.7	30.2 ± 6.9	28.7 ± 5.2	28.5 ± 6.2	26.2 ± 3.9	24.9 ± 4.3	28.8 ± 5.0	27.6 ± 5.7	27.4 ± 4.9	27.6 ± 6.6
Country of birth												
Born USA	2539	2807	599	713	284	448	377	400	1086	507	195	241
Not born USA	323	463	73	84	106	135	493	318	124	90	86	72

Characteristic	White		African American		Hispanic		Asian		High intensity		Multiple race or other	
Total enrolled, screening phase	6518		1616		1024		1624		1976		645	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
FT donation												
FT ever	166	191	85	121	64	105	135	134	0	0	52	61
Not FT ever	2612	2981	570	659	314	469	710	560	1187	586	216	244
Smoking												
Smoke	227	309	95	94	66	60	67	32	62	43	22	23
No smoke	2462	2687	552	682	311	514	778	662	1093	512	241	269
Missing	312	521	99	94	32	41	39	46	166	100	42	48
Iron supplementation												
Yes iron	976	1565	191	322	81	190	194	250	559	422	77	120
No iron	1967	1890	532	540	322	418	672	480	741	220	218	210
Antacid use												
Antacid	487	645	56	97	38	72	55	65	169	90	32	41
No antacid	2499	2844	683	770	369	539	826	673	1139	553	267	291
Hormone use												
Hormone	54	687	13	127	3	105	6	148	17	56	4	87
No hormone	2894	2777	722	733	399	504	864	586	1287	581	291	245
Ever pregnant	0	2222	0	538	0	303	0	280	0	487	0	166

BMI = body mass index; FT = first time.