Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

COVID-19 Source Data and severity definition

VA Informatics and Computing Infrastructure (VINCI) created a COVID-19 Shared Data Resource (SDR) during the April-August 2020 period, documenting information regarding conditions, laboratory measures, medications, and procedures pertaining to the COVID-19 pandemic. This database was hosted by the VA Phenomics Library, Centralized Interactive Phenomics Resource (CIPHER), and provisioned data access to users across the VA healthcare systems. The VA COVID-19 SDR group including the VA National Surveillance Team (NST) provided researchers with curated data extracted from electronic health records (EHR) of veterans, after stripping off identifiable elements, and ensuring data security by requiring restricted access to the data (https://www.hsrd.research.va.gov/covid19.cfm). The phenotyping activity was undertaken centrally by an experienced VA research team in order to ensure consistent applications of dates across the EHR tables, as well as to provide uniform definitions of events preceding and subsequent to COVID-19, in an effort to characterize the trajectory of COVID-19 and other diseases and conditions. VA-wide efforts describing these data are in the public domain[21]. Structured data obtained via CPT and ICD-9 or ICD-10 codes, deposited in the VA Corporate Data Warehouse (CDW), was boosted with rule-based unstructured events recorded in patient notes via natural language processing (NLP), as was described previously for other projects[42]. The purpose of NLP-boosting was to fill gaps in knowledge about the severity of the disease, as well as extract specific dates when procedures (e.g. intubation and extubation) were performed. These curated data were provisioned in the MVP study mart for COVID-19 in VINCI ensuring data security behind the VA firewall.

EHR data for COVID-19 severity and comorbidity assessment was obtained from the time of enrollment at a VA medical center through February 2021. All subjects from this study had their COVID-19 testing performed at the VA using PCR based methods[18]. The index date was

defined as a COVID-19 case date, and for a hospitalized patient this can be the admission date up to 15 days prior to the COVID-19 case date. The MVP Data Core and MVP COVID-19 study team created a COVID-19 Severity Scale variable adapting the WHO COVID-19 Disease Progression Scale[19].

The severity of COVID-19 infection was characterized based on testing positive for COVID-19 by PCR, hospitalization within 30 days of index dates for COVID-19 infection, and severe COVID-19 infection which includes treatment by noninvasive mechanical ventilation (NIV), high flow oxygen supply, extracorporeal membrane oxygenation (ECMO), intubation, pressor support, and dialysis; and death within 30 days of index dates for COVID-19 infection [19]. Based on these criterias, we derived the following binary COVID-19 outcomes: (1) COVID-19 infectivity (COVID-19 positive vs. negative); (2) COVID-19 hospitalization (COVID-19 positive and hospitalized vs. COVID-19 positive but not hospitalized); (3) severe COVID-19 (COVID19 severe cases based on WHO criteria described above vs. testing positive for COVID-19 but not severe); (4) death (death within 30 days of COVID-19 infection vs. all other COVID-19 test positives).

Electronic Health Records on MVP participants

The MVP, a large multi-ethnic genetic biobank of US Veterans supported by the VA office of Research and Development[17], served as the primary cohort analyzed for this COVID-19 study. MVP began enrollment in 2011, with continued enrollment to include > 850,000 Veterans as of mid 2021, with >650,000 genotyped. Blood biospecimens were collected for DNA isolation and genotyping, and the biorepository was linked to Veterans' EHR data, which included diagnosis codes (International Classification of Diseases ninth [ICD-9] and tenth [ICD-10]) revisions, current procedural terminology codes (CPT). Clinical laboratory measures, and demographic, lifestyle, and imaging data[43] were also available for analysis. EHR data captured on participants

from the time of enrollment at a VA healthcare center through September 30, 2019 represented their pre-COVID status.

The single nucleotide polymorphism (SNP) data in the MVP cohort was generated using a custom Thermo Fisher axiom genotyping platform called MVP 1.0. The quality control steps and genotyping imputation using 1000 Genomes cosmopolitan reference panel for the MVP cohort have been previously reported[44]. Harmonized ancestry, race and ethnicity (HARE) is a composite variable derived from a combination of self-reported survey information and genetically derived ancestry [26], was employed as a proxy for population stratification and global ancestry. We focused on three major ancestral groups: African (AFR), Hispanic (HIS), and European (EUR). Either directly genotyped or imputed markers in the beta hemoglobin gene (rs334(T) and rs33930165) were extracted and utilized for association testing. rs334 refers to rs334(T) and will be used interchangeably in this report.

Phenome-wide association study (PheWAS) and Laboratory-wide association study (LabWAS) of pre-existing conditions with Hb alleles

For the PheWAS analysis on rs334 SNP, we used phecodes[45] and laboratory measures from the clinical data available prior to the onset of COVID-19 infection (Sept 2019). For each phecode, individuals who had 2 or more phecodes reported were defined as cases, whereas those with no instance of a phecode were defined as controls. We have previously shown that cases <200 could lead to low statistical power and higher type I error[46], therefore, we applied the threshold of 200 cases in each ancestry and excluded any phecode that was below that limit. This approach resulted in 1,618 (EUR), 1289 (AFR), 994 (HIS), 293 (ASN) phecodes being analyzed. We included 65 common clinical laboratory test measures available on the MVP participants up to March 2019. We used the median from the complete history of laboratory values for each individual. Additionally, for each laboratory measure, we excluded extreme

values that were >3 standard deviations from the mean. Logistic regression was applied to phecode-based models, whereas linear regression was used for laboratory measures implemented in PLINK2[47]. Additionally, Firth regression was applied when the logistic regression model failed to converge for binary outcomes. All the regression models were adjusted for sex, age (at enrollment), quadratic term for age, and the first 20 principal components. Ancestry-specific PheWAS and LabWAS were performed in all four groups. We applied a Bonferroni correction to adjust for multiple hypothesis testing, and the threshold of significance was computed independently in each ancestry group.

Phenotyping of pre- and post-index conditions for COVID-19

Pre-index conditions were extracted by applying natural language processing (NLP) on ICD codes/procedures codes/medications within 2 years prior to the index date of COVID-19 diagnosis. Similar processes were used to extract post-index conditions 60 days after the index date. Pre-index renal impairments included acute renal failure (AKF2yr), prior end stage renal failure (AKIpeakmodifiedstage= priorEndStageRenalDisease), chronic kidney disease (CKD2yr), chronic kidney failure (CKF2yr), and nephrosis (Nephrosis2yr). The post-index conditions we studied included acute kidney failure (AKF60d), acute myocardial infarction (AMI60d) and a worsening trend of renal functions (postindex AKIpeakmodifiedstage= 1, 2, or 3) from a patient cohort with stable renal function pre-index (preindex AKIpeakmodifiedstage= 0).

Stages of Acute Kidney Disease (Akipeakmodifiedstage)

Akitpeakmodifiedstage is defined by the following: we take every creatinine in the index periods of interest and compare with the average baseline creatinine values from the one year baseline period preceding the index period. And then we take the patient's maximum stage.

If creatinine / baseline creatinine >= 3.0 then Stage 3

If creatinine / baseline creatinine >= 2.0 then Stage 2

If creatinine / baseline creatinine >= 1.5 then Stage 1

If creatinine – baseline creatinine >= 0.3 then Stage 1

If a patient is undergoing dialysis in the index period of interest, it is defined as Stage 3.

Stage “PriorESRD” refers to patients that had eGFR values from baseline that averaged <15, or prior history of dialysis or prior history of renal transplant.

Stage “Unknown” is defined as missing either baseline creatinine (results in the one year before index periods of interest) and/or missing the creatinine level in the index period of interest. AKI stage is assigned by comparing baseline to index period of interest so without both values the algorithm will not set an AKI stage

Statistical methods

Firth logistic regression[23,24] implemented with the R package “brglm2” (version 0.7.1)[25] was used to examine the association between Hb alleles and COVID-19 outcomes, and ancestry-specific analyses were performed. All models were adjusted for age, age^2, sex, and 20 ethnicity-specific principal components. Adjusted odds ratios (OR) of each copy of Hb allele were reported for the outcomes in each ethnicity, when applicable. The resulting ORs were meta-analyzed across ethnicity groups using random-effects meta-analysis as implemented in the R package “metafor” (version 2.4-0)[28].

In the analysis of AKF60d, we adjusted for the presence of pre-index renal impairments or a worsening trend of renal functions or End Stage Renal Failure (ESRD) (post-index AKIpeakmodifiedstage= 1,2,or 3 or priorESRD ***plus*** post-index AKIpeakmodifiedstage= unknown were excluded).

In the analysis of a worsening trend of renal functions (postindex AKIpeakmodifiedstage= 1,2,or 3), we only focused on patients with pre-index AKIpeakmodifiedstage=0 and excluded participants of pre-index AKIpeakmodifiedstage= unknown or priorESRD).

For each post-index code, we first tested whether COVID-19 infection modified the effect of the SCT on the condition. If the interaction term was statistically significant at alpha <0.05, then COVID-19 infection was interpreted to be an effect modifier. The final model for inference included the interaction term and four ORs for the condition were reported: 1) SCT+ vs. SCT- in COVID-19 positive patients, SCT+ vs. SCT- in COVID-19 negative patients, 2) COVID-19 positive vs. COVID-19 negative in SCT+ patients, and 3) COVID-19 positive vs. COVID-19 negative in SCT- patients. If the interaction term was not statistically significant at alpha level of 0.05, then the final model for inference did not include the interaction term and two ORs for the condition were reported: SCT+ vs. SCT-, and COVID-19 positive vs. COVID-19 negative.

Mediation analysis

We used counterfactual mediation modeling to investigate whether specific conditions which arose post-COVID-19 in SCT individuals caused mortality. We examined two candidates for potential mediation of the COVID-19 death by the SCT: post-index AKF60d and AMI60d in the AFR ancestral group. We used the mediation framework implemented in R (v3.6.1) (https://www.R-project.org/), mediation package version 4.5.0[48], to estimate the direct and indirect effects for each mediator separately. The mediator model (with the mediator as the dependent variable and SCT as an independent variable) and the outcome model (with death as the dependent variable, SCT as independent variable and the mediator) both adjust for age, quadratic term of age, sex, and first 20 ethnicity-specific principal components. We used 10,000 simulations to report estimates and 95% confidence intervals. Because of the random

component of the mediation analysis, for each postindex condition mediation analysis was performed with 3 different random seeds. They all produce similar estimates.

More details on PheWAS and LabWAS results and discussion

PheWAS

At 1.6% allele frequency of the HbS variant (rs334) among participants of AFR ancestry, we identified 31 phenotypes with significant association ($p_{adjusted} < 1.48 \times 10^{-05}$). In HIS and EUR participants, there were six phenotypic associations ($p_{adjusted} < 1.95 \times 10^{-05}$), and one association ($p_{adjusted} < 3.09 \times 10^{-05}$), respectively (Figure 2, eTable 3).

Fewer conditions were identified among HIS participants, such as chronic kidney disease (OR_{HIS}= 2.3 [1.7 – 3.2], $p_{HIS} = 3.04 \times 10^{-08}$) and hypertensive chronic kidney disease (OR_{HIS} = 2.14 [1.55 - 2.98], $p_{HIS}= 4.5 \times 10^{-06}$). Notably, both these conditions show ~60% more risk among HIS than AFR ancestry participants. However, it is important to note that the sample size of HIS group is much smaller with wider confidence intervals. Among EUR, hereditary hemolytic anemia was the only significant association (phecode: 282; OR = 14.7 [8.11 – 26.6], $p = 8.76 \times 10^{-19}$), which was observed among AFR as well (OR = 10.3 [9.5 – 11.2], $p = 1 \times 10^{-300}$).

LabWAS

We identified 11/64 in EUR, 39/61 in AFR, 17/53 in HIS significant associations with rs334(T). A decrease in mean corpuscular hemoglobin (MCH) levels had the most significant association across all the ancestry groups (Figure 3, eTable 4). Additional associations with several red blood cell traits previously reported in other studies[11,29], such as mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin, hematocrit, and red blood cell width were observed (Figure 3, eTable 4).

Lymphopenia is an adverse prognostic factor for COVID-19 infection at the time of

diagnosis[49,50], and in our study we observed that rs334 associated with a decreased lymphocyte fraction (units are percent) in AFR (beta = -2.68, p = 2.11 x 10^{-133}), HIS (beta = -2.66, p = 7.74 x 10^{-12}), and EUR (beta = -1.95, p = 1.06 x 10^{-05}).

Additional References

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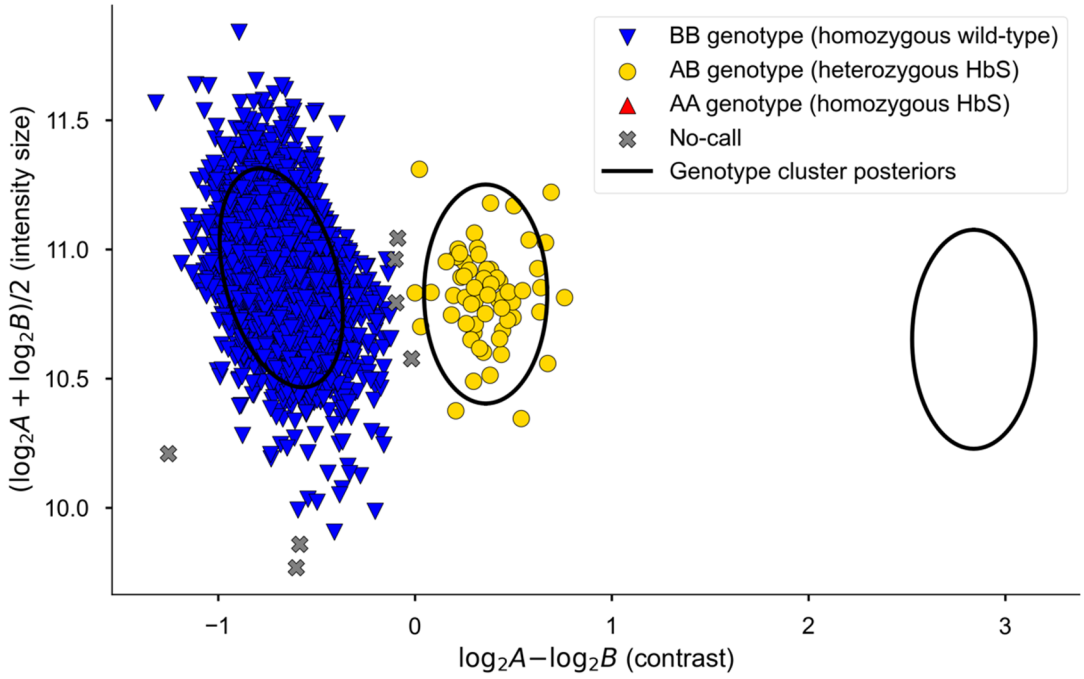
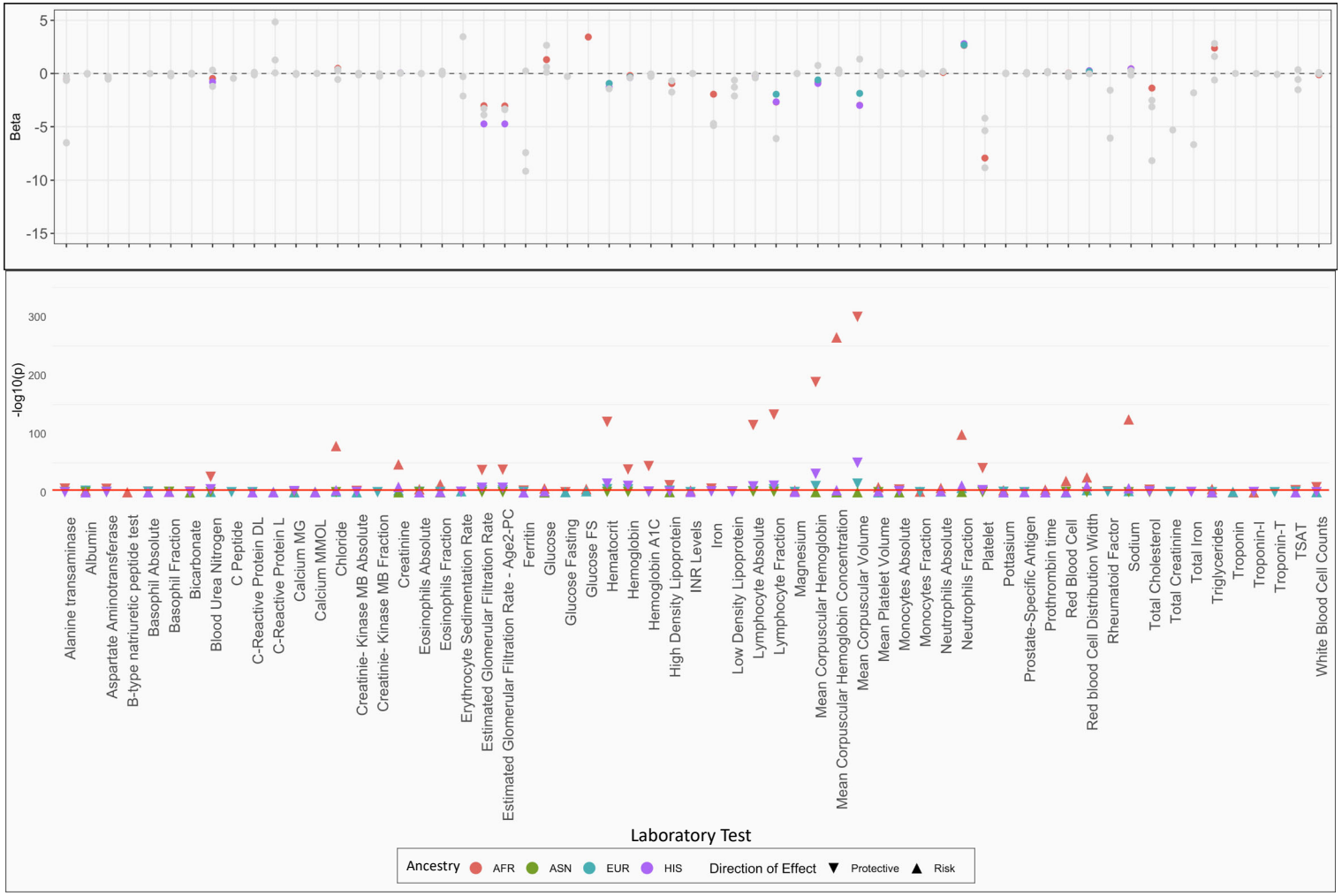
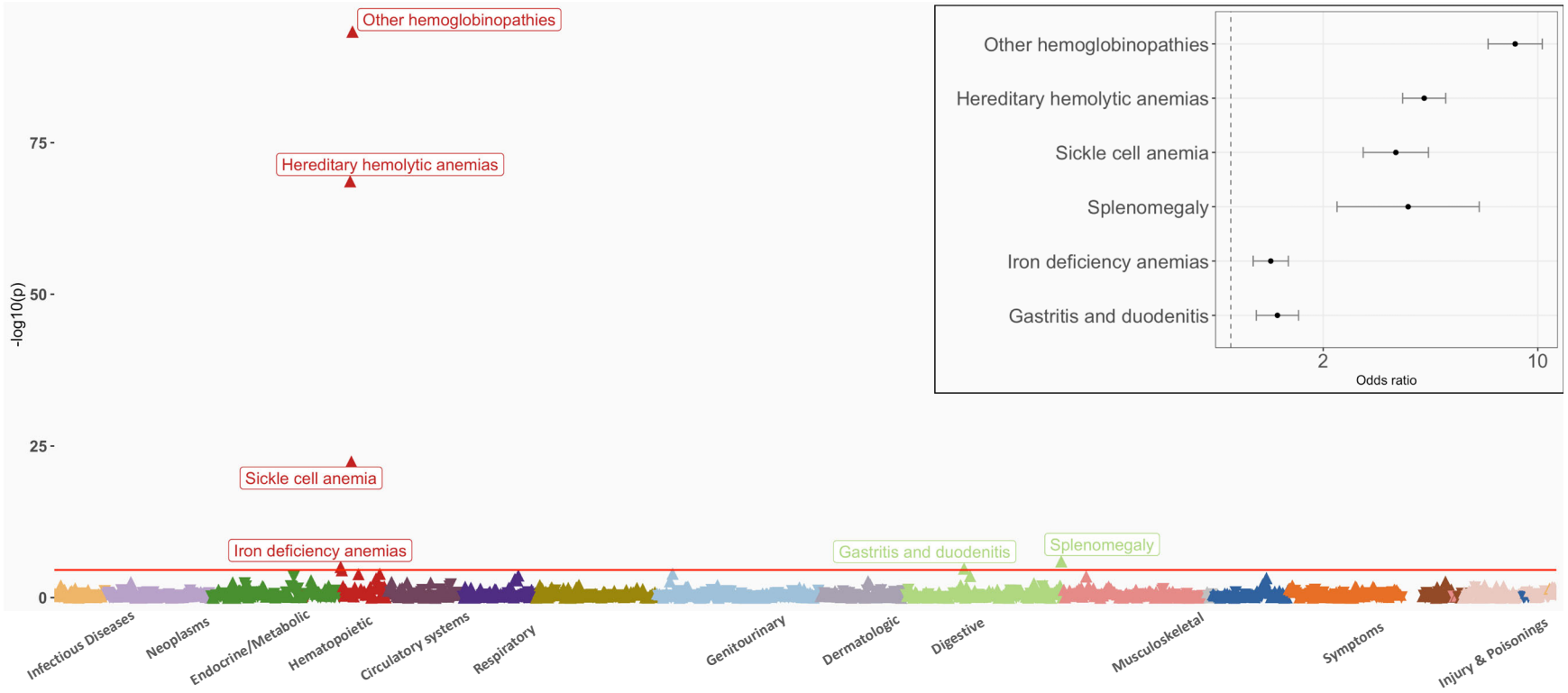
eFigure 1. A hybridization intensity distribution plot of human beta hemoglobin A (HbA, the wild type allele) and hemoglobin S (HbS, the alternative sickle allele rs334) in a representative batch (probeset AX-42810399, MVP 1.0 Axiom Array; out of 161 batches) of 4353 MVP participants. The expected location of a HbS/S homozygote is circled. Review of all 161 batches from MVP revealed there was no HbS/S in the study population.



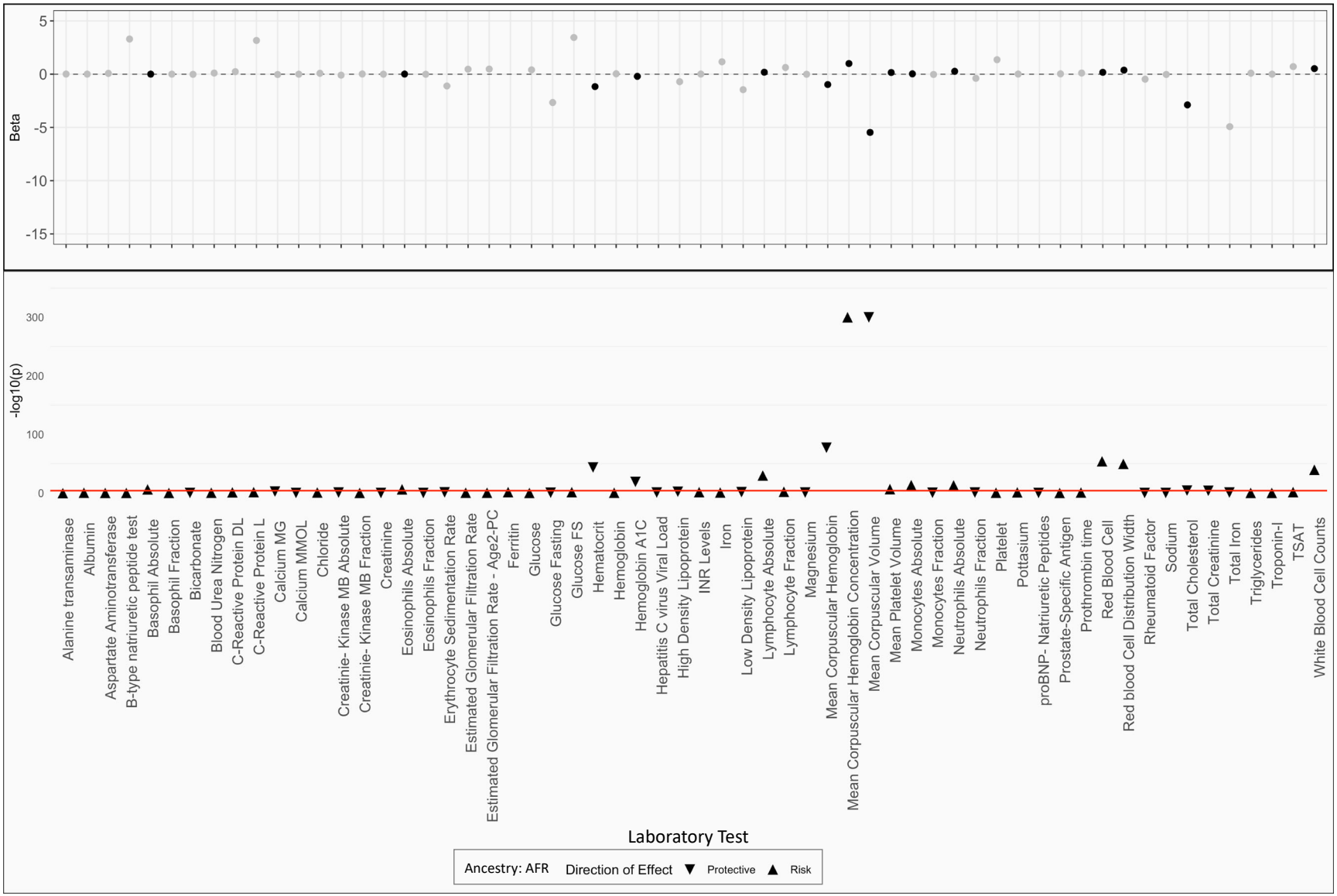
eFigure 2. LabWAS of rs334-T in the MVP. A plot showing the associations of rs334-T and the median value of 65 laboratory measures. a) The panel shows the -log10 (p-value) on the y-axis and lab descriptions on the x-axis. Triangles pointing up have increasing effects, and triangles pointing down have decreasing effects. The colors represent the different ancestry groups. b) The plot shows beta from the regression model for each laboratory measure. The significant results are highlighted in colors corresponding to ancestry groups, and other results are plotted in grey.



eFigure 3. Phenome-wide association studies of the Hb C allele (rs33930165-T) in MVP participants from AFR ancestry.



eFigure 4. Laboratory-wide association studies of the Hb C allele (rs33930165-T) in MVP participants from AFR ancestry.



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- Eastern Oklahoma VA Health Care System (Douglas Ivins, M.D.)
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- James A. Haley Veterans' Hospital (Stephen Mastorides, M.D.)
  13000 Bruce B. Downs Blvd, Tampa, FL 33612
- James H. Quillen VA Medical Center (Jonathan Moorman, M.D., Ph.D.)
  Corner of Lamont & Veterans Way, Mountain Home, TN 37684
- John D. Dingell VA Medical Center (Saib Gappy, M.D.)
  4646 John R Street, Detroit, MI 48201
- Louisville VA Medical Center (Jon Klein, M.D., Ph.D.)
  800 Zorn Avenue, Louisville, KY 40206
- Manchester VA Medical Center (Nora Ratcliffe, M.D.)
  718 Smyth Road, Manchester, NH 03104

- Miami VA Health Care System (Hermes Florez, M.D., Ph.D.)
  1201 NW 16th Street, 11 GRC, Miami FL 33125
- Michael E. DeBakey VA Medical Center (Olaoluwa Okusaga, M.D.)
  2002 Holcombe Blvd, Houston, TX 77030
- Minneapolis VA Health Care System (Maureen Murdoch, M.D., M.P.H.)
  One Veterans Drive, Minneapolis, MN 55417
- N. FL/S. GA Veterans Health System (Peruvemba Sriram, M.D.)
  1601 SW Archer Road, Gainesville, FL 32608
- Northport VA Medical Center (Shing Shing Yeh, Ph.D., M.D.)
  79 Middleville Road, Northport, NY 11768
- Overton Brooks VA Medical Center (Neeraj Tandon, M.D.)
  510 East Stoner Ave, Shreveport, LA 71101
- Philadelphia VA Medical Center (Darshana Jhala, M.D.)
  3900 Woodland Avenue, Philadelphia, PA 19104
- Phoenix VA Health Care System (Samuel Aguayo, M.D.)
  650 E. Indian School Road, Phoenix, AZ 85012
- Portland VA Medical Center (David Cohen, M.D.)
  3710 SW U.S. Veterans Hospital Road, Portland, OR 97239
- Providence VA Medical Center (Satish Sharma, M.D.)
  830 Chalkstone Avenue, Providence, RI 02908
- Richard Roudebush VA Medical Center (Suthat Liangpunsakul, M.D., M.P.H.)
  1481 West 10th Street, Indianapolis, IN 46202
- Salem VA Medical Center (Kris Ann Oursler, M.D.)
  1970 Roanoke Blvd, Salem, VA 24153
- San Francisco VA Health Care System (Mary Whooley, M.D.)
  4150 Clement Street, San Francisco, CA 94121
- South Texas Veterans Health Care System (Sunil Ahuja, M.D.)
  7400 Merton Minter Boulevard, San Antonio, TX 78229
- Southeast Louisiana Veterans Health Care System (Joseph Constans, Ph.D.)
  2400 Canal Street, New Orleans, LA 70119
- Southern Arizona VA Health Care System (Paul Meyer, M.D., Ph.D.)
  3601 S 6th Avenue, Tucson, AZ 85723
- Sioux Falls VA Health Care System (Jennifer Greco, M.D.)
  2501 W 22nd Street, Sioux Falls, SD 57105
- St. Louis VA Health Care System (Michael Rauchman, M.D.)
  915 North Grand Blvd, St. Louis, MO 63106
- Syracuse VA Medical Center (Richard Servatius, Ph.D.)
  800 Irving Avenue, Syracuse, NY 13210
- VA Eastern Kansas Health Care System (Melinda Gaddy, Ph.D.)
  4101 S 4th Street Trafficway, Leavenworth, KS 66048
- VA Greater Los Angeles Health Care System (Agnes Wallbom, M.D., M.S.)
  11301 Wilshire Blvd, Los Angeles, CA 90073
- VA Long Beach Healthcare System (Timothy Morgan, M.D.)
  5901 East 7th Street Long Beach, CA 90822

- VA Maine Healthcare System (Todd Stapley, D.O.)
  1 VA Center, Augusta, ME 04330
- VA New York Harbor Healthcare System (Scott Sherman, M.D., M.P.H.)
  423 East 23rd Street, New York, NY 10010
- VA Pacific Islands Health Care System (George Ross, M.D.)
  459 Patterson Rd, Honolulu, HI 96819
- VA Palo Alto Health Care System (Philip Tsao, Ph.D.)
  3801 Miranda Avenue, Palo Alto, CA 94304-1290
- VA Pittsburgh Health Care System (Patrick Strollo, Jr., M.D.)
  University Drive, Pittsburgh, PA 15240
- VA Puget Sound Health Care System (Edward Boyko, M.D.)
  1660 S. Columbian Way, Seattle, WA 98108-1597
- VA Salt Lake City Health Care System (Laurence Meyer, M.D., Ph.D.)
  500 Foothill Drive, Salt Lake City, UT 84148
- VA San Diego Healthcare System (Samir Gupta, M.D., M.S.C.S.)
  3350 La Jolla Village Drive, San Diego, CA 92161
- VA Sierra Nevada Health Care System (Mostaqul Huq, Pharm.D., Ph.D.)
  975 Kirman Avenue, Reno, NV 89502
- VA Southern Nevada Healthcare System (Joseph Fayad, M.D.)
  6900 North Pecos Road, North Las Vegas, NV 89086
- VA Tennessee Valley Healthcare System (Adriana Hung, M.D., M.P.H.)
  1310 24th Avenue, South Nashville, TN 37212
- Washington DC VA Medical Center (Jack Lichy, M.D., Ph.D.)
  50 Irving St, Washington, D. C. 20422
- W.G. (Bill) Hefner VA Medical Center (Robin Hurley, M.D.)
  1601 Brenner Ave, Salisbury, NC 28144
- White River Junction VA Medical Center (Brooks Robey, M.D.)
  163 Veterans Drive, White River Junction, VT 05009
- William S. Middleton Memorial Veterans Hospital (Robert Striker, M.D., Ph.D.)
  2500 Overlook Terrace, Madison, WI 53705