

Impact of Rare and Common Genetic Variants on Diabetes Diagnosis by Hemoglobin A1c in Multi-Ancestry Cohorts: The Trans-Omics for Precision Medicine Program

Chloé Sarnowski,^{1,35,37,*} Aaron Leong,^{2,3,4,36,37,*} Laura M. Raffield,⁵ Peitao Wu,¹ Paul S. de Vries,⁶ Daniel DiCorpo,¹ Xiuqing Guo,⁷ Huichun Xu,⁸ Yongmei Liu,⁹ Xiuwen Zheng,¹⁰ Yao Hu,¹¹ Jennifer A. Brody,¹² Mark O. Goodarzi,¹³ Bertha A. Hidalgo,¹⁴ Heather M. Highland,¹⁵ Deepti Jain,¹⁰ Ching-Ti Liu,¹ Rakhi P. Naik,¹⁶ Jeffrey R. O'Connell,¹⁷ James A. Perry,¹⁷ Bianca C. Porneala,² Elizabeth Selvin,¹⁸ Jennifer Wessel,^{19,20} Bruce M. Psaty,^{12,21,22} Joanne E. Curran,²³ Juan M. Peralta,²³ John Blangero,²³ Charles Kooperberg,¹¹ Rasika Mathias,^{18,24} Andrew D. Johnson,^{25,26}

(Author list continued on next page)

Hemoglobin A1c (HbA1c) is widely used to diagnose diabetes and assess glycemic control in individuals with diabetes. However, non-glycemic determinants, including genetic variation, may influence how accurately HbA1c reflects underlying glycemia. Analyzing the NHLBI Trans-Omics for Precision Medicine (TOPMed) sequence data in 10,338 individuals from five studies and four ancestries (6,158 Europeans, 3,123 African-Americans, 650 Hispanics, and 407 East Asians), we confirmed five regions associated with HbA1c (*GCK* in Europeans and African-Americans, *HK1* in Europeans and Hispanics, *FN3K* and/or *FN3KRP* in Europeans, and *G6PD* in African-Americans and Hispanics) and we identified an African-ancestry-specific low-frequency variant (rs1039215 in *HBG2* and *HBE1*, minor allele frequency (MAF) = 0.03). The most associated *G6PD* variant (rs1050828-T, p.Val98Met, MAF = 12% in African-Americans, MAF = 2% in Hispanics) lowered HbA1c (−0.88% in hemizygous males, −0.34% in heterozygous females) and explained 23% of HbA1c variance in African-Americans and 4% in Hispanics. Additionally, we identified a rare distinct *G6PD* coding variant (rs76723693, p.Leu353Pro, MAF = 0.5%; −0.98% in hemizygous males, −0.46% in heterozygous females) and detected significant association with HbA1c when aggregating rare missense variants in *G6PD*. We observed similar magnitude and direction of effects for rs1039215 (*HBG2*) and rs76723693 (*G6PD*) in the two largest TOPMed African American cohorts, and we replicated the rs76723693 association in the UK Biobank African-ancestry participants. These variants in *G6PD* and *HBG2* were monomorphic in the European and Asian samples. African or Hispanic ancestry individuals carrying *G6PD* variants may be underdiagnosed for diabetes when screened with HbA1c. Thus, assessment of these variants should be considered for incorporation into precision medicine approaches for diabetes diagnosis.

Introduction

Hemoglobin A1c (HbA1c) is a convenient indirect measure of long-term exposure to blood glucose concentrations. HbA1c estimates the proportion of glycosylated hemoglobin, an irreversible chemical modification of the hemoglobin

molecule by blood glucose, in the blood.¹ As HbA1c reflects average ambient glycemia over the previous two to three months, the life of an erythrocyte, it is a commonly used as a test to diagnose diabetes (MIM: 125853 and MIM: 222100) and estimate glycemic control in individuals with diabetes. However, non-glycemic variation in HbA1c due

¹Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA; ²Division of General Internal Medicine, Massachusetts General Hospital, Boston 02114, MA USA; ³Department of Medicine, Harvard Medical School, Boston, MA 02115, USA; ⁴Programs in Metabolism and Medical & Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA; ⁵Department of Genetics, University of North Carolina, Chapel Hill, NC 27514, USA; ⁶Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX 77030, USA; ⁷Institute for Translational Genomics and Population Sciences, LABioMed and Department of Pediatrics at Harbor-UCLA Medical Center, Torrance, CA 90502, USA; ⁸Division of Endocrinology, Diabetes and Nutrition, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA; ⁹Department of Epidemiology & Prevention, Wake Forest School of Medicine, Winston-Salem, NC 27101, USA; ¹⁰Department of Biostatistics, University of Washington, Seattle, WA 98195, USA; ¹¹Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98108, USA; ¹²Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA 98195, USA; ¹³Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA; ¹⁴University of Alabama at Birmingham, Department of Epidemiology, Birmingham, AL 35294, USA; ¹⁵Department of Epidemiology, UNC Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; ¹⁶Division of Hematology, Department of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA; ¹⁷University of Maryland School of Medicine, Baltimore, MD 21201, USA; ¹⁸Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA; ¹⁹Department of Epidemiology, Indiana University Fairbanks School of Public Health, Indianapolis, IN 46202, USA; ²⁰Department of Medicine and Diabetes Translational Research Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA; ²¹Kaiser Permanente Washington Health Research Institute, Seattle, WA 98101, USA; ²²Departments of Epidemiology and Health Services, University of Washington, Seattle, WA 98195, USA; ²³Department of Human Genetics and South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Brownsville, TX 78520, USA; ²⁴GeneSTAR Research Program, Department of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA; ²⁵National Heart, Lung, and Blood Institute and Boston University's Framingham Heart Study, Framingham MA 01702, USA; ²⁶Population Sciences Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20814, USA; ²⁷Department of Epidemiology, University of Washington, Seattle, WA 98195, USA; ²⁸Geriatrics Research

(Affiliations continued on next page)



Alexander P. Reiner,^{11,27} Braxton D. Mitchell,^{8,28} L. Adrienne Cupples,^{1,25} Ramachandran S. Vasan,^{25,29,30} Adolfo Correa,^{31,32} Alanna C. Morrison,⁶ Eric Boerwinkle,^{6,33} Jerome I. Rotter,⁷ Stephen S. Rich,³⁴ Alisa K. Manning,^{2,3,4} Josée Dupuis,^{1,25,37} James B. Meigs,^{2,3,4,37} TOPMed Diabetes Working Group, TOPMed Hematology Working Group, TOPMed Hemostasis Working Group, and National Heart, Lung, and Blood Institute TOPMed Consortium

to differences in erythrocyte turnover can influence how accurately HbA1c reflects underlying glycemia.²

We previously conducted a trans-ethnic genome-wide association study (GWAS) meta-analysis of HbA1c in 159,940 individuals from four ancestries (European, African, East Asian, and South Asian). We identified 60 common (minor allele frequency [MAF] greater than 5%) genetic variants associated with HbA1c, of which 19 were classified as “glycemic” and 22 as “erythrocytic” based on the probable biological mechanism through which they appeared to influence HbA1c levels.³ Genetic variants affecting HbA1c via erythrocyte biological pathways may lead to diagnostic misclassification of ambient glycemia and thus of diabetes status. HbA1c GWAS have so far focused on genetic variants imputed to HapMap,⁴ and therefore genetic discovery efforts have been focused on common variants. Low-frequency (0.5% < MAF < 5%) and rare (MAF < 0.5%) genetic variants and their associated impact on the diagnostic accuracy of HbA1c have not been systematically examined but are suspected to occur.^{5,6} Further, previous GWAS have shown that the combined effect of HbA1c-related common variants causes differences in HbA1c that were three times greater in individuals of African ancestry compared with those of European ancestry (0.8 [%-units] versus 0.25 [%-units]).³ This relatively large difference in HbA1c was mainly driven by a single African-ancestry-specific missense variant (rs1050828-T, p.Val98Met) in the Glucose-6-phosphatase Dehydrogenase gene (*G6PD* [MIM: 305900]), which causes G6PD deficiency (MIM: 300908).^{7,8} As the genetic architecture of HbA1c appears to differ by ancestry, as does type 2 diabetes risk, it is imperative to understand the genetic basis of HbA1c in different ancestral groups to ensure that large-effect ancestry-specific variants, similar to the *G6PD* variant, are uncovered.⁹

We sought to identify common, low frequency, and rare genetic variants (single-nucleotide variants [SNVs] and structural variations) associated with HbA1c through association analyses in diabetes-free individuals from four ancestries by using whole-genome sequencing (WGS) data from the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) Trans-Omics

for Precision Medicine (TOPMed) program. We hypothesized that variants that are in the low frequency spectrum and that have relatively large effects will be detected even with modest sample sizes. To uncover additional distinct erythrocytic variants associated with HbA1c, we performed gene-based tests and fine-mapping by using sequential conditional association analyses of erythrocytic loci that reached genome-wide significance in this study. We then sought replication of genome-wide and sub-genome-wide TOPMed signals in participants from the UK Biobank who had European, African, or Asian ancestry.

Material and Methods

Populations and Participants

We included in our analyses 10,338 TOPMed participants without diabetes from five cohorts: the Old Order Amish study (n = 151), the Atherosclerosis Risk in Communities Study (ARIC, n = 2,415), the Framingham Heart Study (FHS, n = 2,236), the Jackson Heart Study (JHS, n = 2,356), and the Multi-Ethnic Study of Atherosclerosis (MESA, n = 3,180). These participants represent four ancestry groups: Europeans (EA, n = 6,158), African-Americans (AA, n = 3,123), Hispanics or Latinos (HA, n = 650), and East Asians (AS, n = 407) (Table S1). Descriptions of each cohort are available in the Supplemental Data. Diabetes was defined as fasting glucose (FG) ≥ 7 mmol/L after ≥ 8 h, HbA1c ≥ 6.5%-units, 2 h glucose by an oral glucose tolerance test ≥ 11.1 mmol/L, non-fasting glucose ≥ 11.1 mmol/L, physician-diagnosed diabetes, self-reported diabetes, or use of an antidiabetic medication. Measures of FG and 2 h glucose made in whole blood were corrected to plasma levels using the correction factor of 1.13. Individual studies applied further sample exclusions where applicable, including pregnancy and type 1 diabetes status.

Measurement of HbA1c and Erythrocytic Traits

The National Glycohemoglobin Standardization Program (NGSP) certified assays¹⁰ used to measure HbA1c in each cohort are indicated in Table S2. HbA1c was expressed in NGSP %-units. Measurements for red blood cell (RBC) count (× 10¹²/L), hemoglobin (HB; g/dL), hematocrit (HCT; %), mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC; g/dL), and red blood cell

and Education Clinical Center, Baltimore Veterans Administration Medical Center, Baltimore, MD 21201, USA; ²⁹Section of Preventive Medicine and Epidemiology, Evans Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA; ³⁰Whitaker Cardiovascular Institute and Cardiology Section, Evans Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA; ³¹Departments of Medicine, Pediatrics, and Population Health Science, University of Mississippi Medical Center, Jackson, MS 39216, USA; ³²The Jackson Heart Study, Jackson, MS 39213, USA; ³³Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA; ³⁴Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908, USA; ³⁵Present address: Department of Biostatistics, Boston University School of Public Health, 801 Massachusetts Avenue, MA 02118, Boston, USA; ³⁶Present address: Division of General Internal Medicine, Massachusetts General Hospital, 100 Cambridge St 16th Floor, MA 02114, Boston, USA

³⁷These authors contributed equally to this work

*Correspondence: chloesar@bu.edu (C.S.), ASLEONG@partners.org (A.L.)

<https://doi.org/10.1016/j.ajhg.2019.08.010>

distribution width (RDW; %) were obtained from complete blood count panels performed using standard assays.

Whole-Genome Sequencing

The NHLBI TOPMed program provided WGS, performed at an average depth of 38 × by several sequencing centers (New York Genome Center, Broad Institute of MIT and Harvard, University of Washington Northwest Genomics Center, Illumina Genomic Services, Macrogen Corporation, and Baylor Human Genome Sequencing Center) using DNA from blood. Details regarding the laboratory methods, data processing, and quality control are described on the TOPMed website (see URL in the [Web Resources](#) section) and in documents included in each TOPMed accession released on the database of Genotypes and Phenotypes (dbGaP). Processing of whole genome sequences was harmonized across genomic centers using a standard pipeline.¹¹

We used TOPMed freeze 5b that comprised 54,508 samples. We performed variant discovery and genotype calling jointly by using the GotCloud pipeline across TOPMed parent studies for all samples. A support vector machine quality filter was trained using known variants (positive training set) and Mendelian-inconsistent variants (negative training set). The TOPMed data coordinating center performed additional quality control checks for sample identity issues including pedigree errors, sex discrepancies, and genotyping concordance. After site level filtering, TOPMed freeze 5b consisted of ~438 million SNVs and ~33 million short insertion-deletion variants. Read mapping was done using the 1000 Genomes Project reference sequence versions for human genome build GRCh38.

The study was approved by the appropriate institutional review boards (IRBs) and informed consent was obtained from all participants.

Statistical Analyses in TOPMed

We first pooled the data across all of the studies, and then we performed pooled WGS association analysis of HbA1c in each ancestry separately using GENetic ESTimation and Inference in Structured samples (GENESIS, see URL in the [Web Resources](#) section)¹² on the Analysis Commons.¹³ We used linear mixed effect models to test the association of HbA1c with the genetic variants individually while adjusting for sex and age at HbA1c measurement and study, and allowing for heterogeneous variance across study groups. We accounted for relatedness within each ancestry by using an empirical kinship matrix (Genetic Relatedness Matrix [GRM]) calculated globally with Mixed Model Analysis for Pedigrees and Populations (MMAP, see URL in the [Web Resources](#) section)^{14,15} and using the pooled sample of all TOPMed freeze 5b participants (54k participants), and variants with an MAF ≥ 0.001 (totaling ~26.5M variants), without linkage disequilibrium (LD) pruning. We then meta-analyzed ancestry-specific results.

We excluded from our single-variant analyses variants with a minor allele count (MAC) of less than 20 across the combined samples. We used a significance threshold of $p < 2 \times 10^{-8}$ to report an association as genome-wide significant for common, low-frequency, and rare genetic variants, which was slightly more stringent than the widely adopted p value threshold of 5×10^{-8} in GWAS, based on estimations for genome-wide significance for WGS studies in UK10K.^{16,17} For the X chromosome, genotypes were coded as 0 and 2 for males and 0, 1, and 2 for females, and sex-stratified analyses (analyses conducted separately in males

and females) were also performed. We performed additional analyses in AA, adjusted on the sickle cell trait (SCT) variant rs334, as well as haplotype analyses with this variant, using the R package `haplo.stats`.

Because loci that influence HbA1c through erythrocytic mechanisms are expected to cause nonglycemic variation in HbA1c, we sought to classify HbA1c-associated loci as “glycemic” or “erythrocytic” using mediation analyses on FG, HB, MCV, MCH, or MCHC in TOPMed. If the HbA1c-variant association effect size decreased by more than 25% when we added FG to the regression model, the variant was classified as “glycemic.” If a variant was not glycemic, and if the effect size decreased by more than 25% upon adding HB, MCV, MCH, or MCHC to the regression model, the variant was classified as “erythrocytic.” If association effect sizes were unchanged by or increased after mediation adjustment, then the variant was considered “unclassified.”

If HbA1c-associated variants remained unclassified by the mediation analyses, we used association analysis results with FG to classify them as glycemic ($p < 0.005$), and association analysis results with erythrocytic traits (HB, MCV, MCH, MCHC, RBC, HCT, or RDW) to classify them as erythrocytic ($p < 0.005$). Association results for FG were obtained from a TOPMed freeze 5b pooled association analysis performed using a linear mixed effect model in GENESIS, adjusted on age, age squared, sex, body mass index (BMI), and self-reported ancestry ($n = 26,883$). Association results for erythrocytic traits were obtained from several sources. We first used TOPMed freeze 5b single-variant association analyses performed using a score test in GENESIS and adjusted for age, sex, and study with a GRM while allowing for heterogeneous variance across study groups ($n = 25,080$). The number of individuals included per study in each analysis is available in [Table S3](#). Analyses were performed at the University of Washington using the TOPMed pipeline. We also used association results from published GWAS in European (UK Biobank and INTERVAL studies; $n = 173,480$),¹⁸ Hispanic (Hispanic Community Health Study and Study of Latinos [HCHS/SOL]; $n = 12,502$),¹⁹ and African American participants (Continental Origins and Genetic Epidemiology [COGENT] Network [$n \sim 16,500$]²⁰ and the Candidate gene Association Resource (CARE) Project [$n = 7,112$]),²¹ and exome genotyping in 130,273 multi-ethnic individuals.²²

For loci classified as erythrocytic, we performed sequential conditional analyses to determine the number of distinct signals in each region. The regions were defined based on LD plots. We used a Bonferroni correction for the number of genetic variants in the region with $MAC \geq 20$ to define a signal as distinct. We also performed gene-based and burden tests in GENESIS using a different selection of rare genetic variants ($MAF \leq 1\%$) based on functional annotations (missense, high confidence loss-of-function, or synonymous variants).

Results from each analysis were combined across ancestries or between males and females by meta-analysis using METAL.²³ The heterogeneity test between males and females was calculated using the following formula:

$$\frac{(\beta_{\text{males}} - \beta_{\text{females}})^2}{SE_{\text{males}}^2 + SE_{\text{females}}^2 - 2 \times r \times SE_{\text{males}} \times SE_{\text{females}}}$$

This assessed the difference in effect sizes between males (β_{males}) and females (β_{females}) while accounting for correlation (r) between male and female statistics due to relatedness and was calculated outside of METAL. LD calculations in the TOPMed data and regional plots were done, within ancestry group, using the Omics Analysis, Search and Information System (OASIS, see

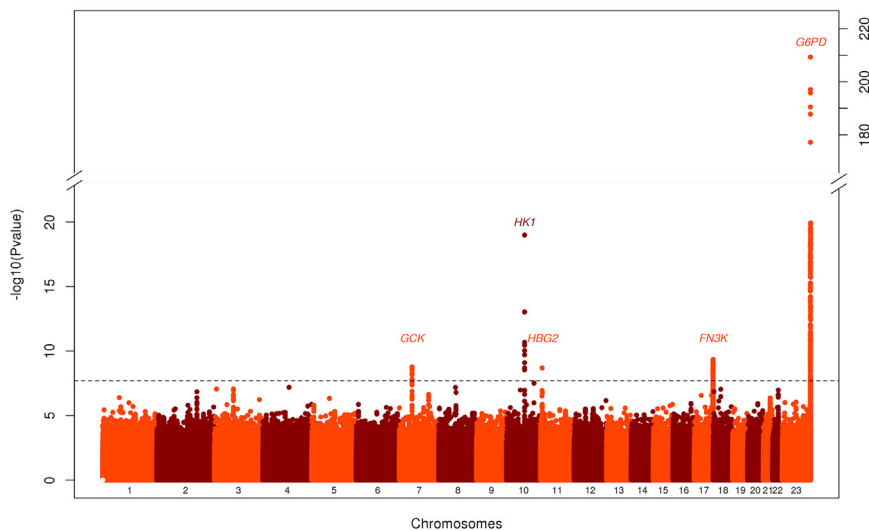


Figure 1. Manhattan Plot of the Meta-Analysis of HbA1c in Non-Diabetic Individuals in TOPMed Cohorts

The $-\log_{10}$ (p value) for each single-nucleotide variant on the y axis is plotted against the build 38 genomic position on the x axis (chromosomal coordinate). The dashed horizontal line indicates the genome-wide significance threshold of $p = 2 \times 10^{-8}$. The y axis was truncated for ease of interpretation.

URL in the [Web Resources](#) section). Functional annotations were performed using the WGS Annotator (WGSAnnot).²⁴

Statistical Analyses in the UK Biobank

We used the UK Biobank (UKBB, see URL in the [Web Resources](#) section), a prospective cohort study with deep genetic and phenotypic data collected on approximately 500,000 individuals aged between 40 and 69 at recruitment from across the United Kingdom, as an independent sample for external replication of our findings. The centralized analysis of the genetic data, including genotype quality, population structure and relatedness of the genetic data, and efficient phasing and genotype imputation, has been described extensively elsewhere.²⁵ Two similar arrays were used for genotyping (Applied Biosystems UK Biobank Lung Exome Variant Evaluation and UK Biobank Axiom Arrays) and pre-phasing was performed using markers present on both arrays. Phasing on the autosomes was carried out using SHAPEIT3 and 1000 Genomes phase 3 panel to help with the phasing of non-European ancestry samples. Imputations were carried out using the IMPUTE4 program with the Haplotype Reference Consortium (HRC) reference panel or with a merged UK10K and 1000 Genomes phase 3 reference panel. For chromosome X, haplotype estimation and genotype imputation were carried out separately on the pseudo-autosomal and non-pseudo-autosomal regions.

We identified UKBB African-ancestry participants using the following three self-reported ethnicities: “Caribbean,” “African,” and “Any other Black background.” We identified UKBB European-ancestry participants using the following three self-reported ethnicities: “White,” “British,” and “Irish.” We identified UKBB Asian-ancestry participants using the “Chinese” self-reported ancestry. We excluded participants with diabetes defined by the use of antidiabetic medication, self-reported physician diagnosis, $FG \geq 7$ mmol/L, or non-fasting glucose ≥ 11.1 mmol/L or HbA1c $\geq 6.5\%$ -units. Because African-ancestry UKBB participants were admixed, we generated principal components (PCs) using smartPCA^{23,24} (see URL in the [Web Resources](#) section) based on 72,300 common genetic variants in low LD selected with PLINK (see URL in the [Web Resources](#) section).²⁵ For each PC, ethnic outliers lying more than 6 SD away from the mean were excluded. Additionally, we excluded samples with high heterozygosity and

high missing rate, sex aneuploidy, or different genomic and stated sex. We used the R package Scalable and Accurate Implementation of Generalized mixed model (SAIGE, see URL in the [Web Resources](#) section)²⁶ and linear mixed effect models to evaluate the association of TOPMed genome-wide and sub-genome-wide signals with HbA1c, adjusting for age and sex, and using a GRM. We also used UKBB exome sequence data available in 50K participants to check the concordance of genotypes with imputed data for rare variants.

Results

HbA1c-Associated Regions Using WGS

We included in our analyses 10,338 TOPMed participants representing four ancestry groups: EA ($n = 6,158$), AA ($n = 3,123$), HA ($n = 650$), and AS ($n = 407$; [Table S1](#)). TOPMed studies were composed of middle- to older-aged participants of EA, AA, HA, or AS ancestry with comparable mean HbA1c, mean fasting glucose (FG), and mean hemoglobin (HB, [Table S4](#)). A total of 13,079,661 variants (EA), 21,443,543 variants (AA), 9,567,498 variants (HA), and 6,567,324 variants (AS) passed filters and were included in the analyses. QQ-plots and Manhattan plots of WGS associations with HbA1c from ancestry-specific analyses and the meta-analysis are provided in [Figure 1](#) and [Figures S1](#) and [S2](#). Using a significance threshold of $p < 2 \times 10^{-8}$ to report an association as genome-wide significant, we detected five regions associated with HbA1c, including one locus that had not been identified in the trans-ethnic GWAS meta-analysis of HbA1c³ (a low-frequency AA-specific variant, rs1039215 in *HBG2* [MIM: 142250] and *HBE1* [MIM: 142100], MAF = 0.03). Regional plot for the *HBG2* and *HBE1* locus is provided in [Figure S3](#). The four other SNVs were located in regions previously identified as associated with HbA1c in trans-ethnic meta-analyses³: rs2971670 in *GSK* (MIM: 138079) on chromosome 7 ($P_{\text{meta}} = 1.7 \times 10^{-9}$, mainly associated in EA and AA, $r^2 = 0.59$, $D' = 1$ with rs4607517, the index SNV in published GWAS), rs17476364 in *HK1* (MIM: 142600) on chromosome 10 ($P_{\text{meta}} = 3.1 \times 10^{-21}$, mainly associated in EA and HA, $r^2 = 0.10$, $D' = 1$ with rs10823343, $r^2 = 0.18$, $D' = 0.50$ with rs4745982, index SNVs in published

Table 1. Most Associated Single Nucleotide Variants in the Regions Detected at the Genome-Wide Level ($p < 2 \times 10^{-6}$) by Ancestry and in the Meta-Analysis of HbA1c in Non-Diabetic Individuals in TOPMed Cohorts

MarkerID ^a	rsID	Closest Gene	Ref/Alt Allele	Meta-Analysis						Europeans (n = 6,158)			African-Americans (n = 3,123)			Hispanics (n = 650)			Asians (n = 407)			
				Beta	SE	p Value	AAF ^b	Beta	SE	p Value	AAF ^b	Beta	SE	p Value	AAF ^b	Beta	SE	p Value	AAF ^b	Beta	SE	p Value
7:44186502	rs2971670	GCK	C/T	0.04	0.01	1.7E-09	0.18	0.03	0.01	1.6E-05	0.18	0.06	0.01	1.6E-04	0.21	0.05	0.03	0.04	0.19	0.04	0.03	0.20
10:69334748	rs17476364	HK1	T/C	-0.09	0.01	3.1E-21	0.10	-0.08	0.01	3.2E-18	0.02	-0.09	0.04	0.04	0.06	-0.15	0.04	5.4E-04	-	-	-	-
11:5464062	rs1039215	HGB2 and HBE1	A/G	-0.21	0.04	2.0E-09	-	-	-	-	0.03	-0.21	0.04	2.0E-09	-	-	-	-	-	-	-	-
17:82739582	rs113373052	FN3K	C/T	0.03	0.01	4.5E-10	0.32	0.03	0.01	4.0E-08	0.29	0.02	0.01	0.06	0.39	0.03	0.02	0.15	0.49	0.05	0.02	0.04
X:154536002	rs1050828	G6PD	C/T	-0.41	0.01	5.1E-210	-	-	-	-	0.12	-0.41	0.01	8.4E-205	0.02	-0.37	0.07	8.3E-07	-	-	-	-

^aMarkerID is defined as Chromosome: Position. Positions are indicated on Build 38.

^bAAF: effect (alternate) allele frequency

GWAS), rs113373052 in *FN3K* (MIM: 608425) on chromosome 17 ($P_{meta} = 4.5 \times 10^{-10}$, associated in EA, $r^2 = 0.92$, $D' = 0.99$, with rs1046896, index SNV in published GWAS), and rs1050828 in *G6PD* on chromosome X ($P_{meta} = 5.1 \times 10^{-210}$, associated in AA and HA, monomorphic in the other ancestries).³ The top SNV or structural variant in each region detected at the genome-wide threshold ($p < 2 \times 10^{-8}$) is indicated in Table 1, and the top one detected at the sub-genome-wide threshold ($p < 5 \times 10^{-7}$) is indicated in Table S5. Genetic association results (summary statistics) are available in the Type 2 Diabetes Knowledge Portal (see URL in the Web Resources section).

Classification of HbA1c-Associated Loci Based on Their Biological Pathways

Because loci that influence HbA1c through erythrocytic pathways are expected to cause nonglycemic variation in HbA1c, we sought to classify HbA1c-associated loci as glycemic or erythrocytic through the use of mediation analyses in TOPMed and association analyses with glycemic and erythrocytic traits in TOPMed or using results from the literature. Mediation analyses and look-up in WGS analysis of FG in TOPMed classified the *GCK* variants as glycemic (Tables S6–S8). Association analyses with erythrocytic traits in published GWAS and in TOPMed showed that the rs17476364-C allele, in the *HK1* gene, was positively associated with HB, MCV, MCH, MCHC, red blood cell count (RBC), HCT, and RDW in Europeans¹⁸ and that the rs1050828-T allele, in the *G6PD* gene, was positively associated with MCH in African-Americans and MCV in African-Americans and Hispanics or Latinos and negatively associated with HCT and HB in African-Americans and RBC and RDW in African-Americans and Hispanics or Latinos^{19–21} (Tables S6, S8, and S9). Results of the mediation and association analyses in TOPMed are available in Tables S6 and S8 for the genome-wide variants and in Tables S7 and S10 for the sub-genome variants. Association results with erythrocytic traits from published GWAS are available in Table S9. Among the 26 variants meeting the sub-genome-wide threshold, four were common and 22 were low frequency or rare. Two of the common HbA1c-associated variants were reported previously:³ the glycemic variant at *G6PC2* (MIM: 608058) and the erythrocytic variant at *TMPRSS6* (MIM: 609862). Among the genome-wide significant loci, *HK1* (EA) and *G6PD* (AA and HA) were classified as erythrocytic, as was the low-frequency variant rs1039215 in *HGB2* and *HBE1*, which was negatively associated with HB, HCT, MCV, and MCH. The significant *FN3K* variant was unclassified.

Characterization of Distinct Signals at Erythrocytic HbA1c-Associated Loci

For loci classified as erythrocytic (*HK1*, *HGB2* and *HBE1* and *G6PD*), we performed sequential conditional analyses to detect distinct signals in addition to the top SNV. The

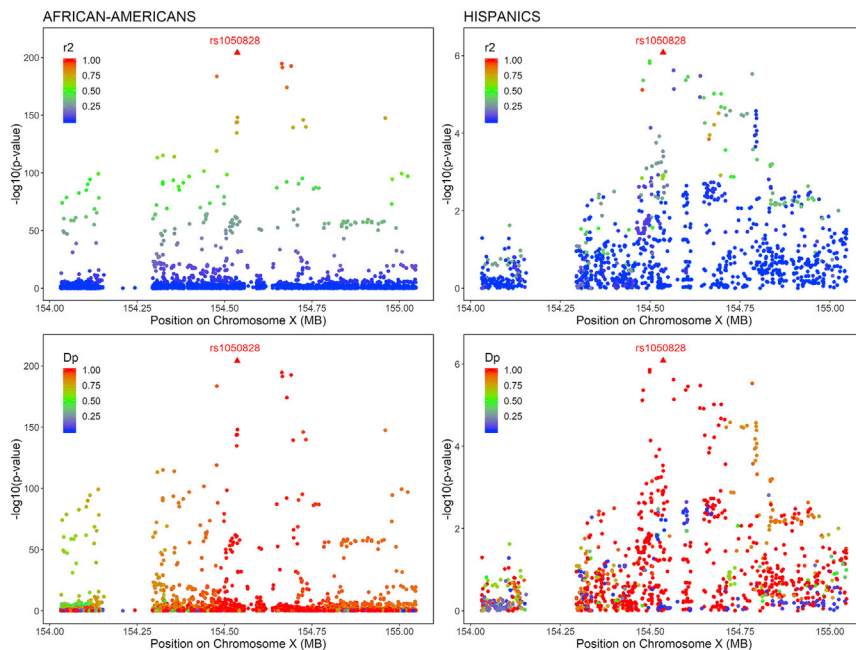


Figure 2. Regional HbA1c Association Plots in the *G6PD* Region in Non-Diabetic African-Americans and Hispanics in TOPMed Cohorts

Association plots are displayed separately in African-Americans (left side) and in Hispanics (right side). Single-nucleotide variants are plotted with their p values ($-\log_{10}$ values, y axis) as a function of build 38 genomic position on chromosome X (x axis). The local linkage disequilibrium (LD) structure (r^2 —top or D' —bottom) between the top associated single-nucleotide variant, rs1050828 (red triangle), and correlated proxies is indicated in color according to a blue-to-red scale from $r^2/D' = 0$ to 1, and this local LD structure was calculated separately in non-diabetic African-American and Hispanic TOPMed cohorts.

regions were defined, based on LD plots, as ± 60 kb region around *HK1*, ± 250 kb region around *HBG2* and *HBE1*, and ± 500 kb region around *G6PD* (Figures S3–S5). We did not detect secondary associations in the *HK1* and *HBG2* and *HBE1* regions at a threshold of 4.3×10^{-5} and 8.5×10^{-6} respectively. In the *G6PD* region, the top SNV (rs1050828, p.Val98Met, T-allele frequency 12% in AA, 2% in HA, and 0% in EA and AS) was associated with lower HbA1c in both AA and HA. This SNV accounted for 23% of HbA1c variance in AA and 4% in HA. The LD in the *G6PD* region is complex, with a strong haplotype effect in AA and HA (Figure 2). By performing conditional analyses on the top SNV (rs1050828), we were able to detect an additional rare signal (G-allele frequency 0.5%) in AA (rs76723693, p.Leu353Pro, $B_{\text{cond}} = -0.50$, $P_{\text{cond}} = 2.8 \times 10^{-15}$) that was distinct from rs1050828 ($r^2 = 0.0006$, $D' = 1$, Figure 3). The threshold to detect an additional signal was fixed to 1.7×10^{-5} in AA and 4.5×10^{-5} in HA. The three other SNVs located in the same region that were sub-genome-wide significant in the main analysis (rs143745197, rs184539426, and rs189305788) were not significant after adjusting for both rs1050828 and rs76723693. We detected significant or suggestive associations by using gene-based ($p = 9.7 \times 10^{-11}$) and burden tests ($p = 4.7 \times 10^{-5}$) when aggregating 15 missense rare variants with $\text{MAF} \leq 1\%$ in *G6PD* (Table S11). Because association from SKAT was more significant than the association of rs76723693 from single-SNV association analysis, it suggested that several rare missense variants in *G6PD* were associated with HbA1c. We performed additional association and conditional analyses in *G6PD* for missense variants with $10 < \text{MAC} < 20$. In addition to rs76723693, one rare missense variant (rs5030872, $\text{MAF} = 0.002$) was suggestively associated with HbA1c ($B = -0.64$, $p = 3.4 \times 10^{-6}$)

and became significantly associated with HbA1c when we adjusted for both rs1050828 and rs76723693 ($B_{\text{cond}} = -0.71$, $P_{\text{cond}} = 1.3 \times 10^{-9}$).

The *HBG2* and *HBE1* variant rs1039215 ($p = 2.0 \times 10^{-9}$) is located at 327 kb from the SCT variant rs334 ($p = 2.9 \times 10^{-8}$). A recent paper reported rs1039215 to be in LD ($r^2 > 0.2$) with the SCT variant (rs334, $r^2 = 0.24$, $D' = -0.83$) and correlated with *HBG2* gene expression in whole blood.²⁷ To determine whether rs1039215 was distinct from rs334, we calculated LD and performed conditional analyses. In TOPMed, rs1039215 was in modest LD with rs334 ($r^2 = 0.30$, $D' = 0.88$, Figure S3), and conditioning on rs334 attenuated its association (decrease in p value from 2.0×10^{-9} to 2.0×10^{-3} , Table S12). These results suggest that the association of rs1039215 with HbA1c may be explained by the SCT rs334 variant. Nevertheless, the haplotype rs334-A and rs1039215-G was more significantly associated with HbA1c than were the haplotypes rs334-A and rs1039215-A and rs334-T and rs1039215-G (Table S13). Thus, the association of the variant rs1039215 in *HBG2* and *HBE1* could be partially distinct from the known association at the SCT variant. In our AA sample, 12 individuals each carried two copies of the rs1050828-T allele and at least one copy of the rs1039215-G allele and had a mean HbA1c of 4.60 (0.41) versus 5.65 (0.39) for the 2,372 individuals who carried no risk allele at the two variants (Table S14).

HbA1c-Lowering Effects of *G6PD* Variants in AA and HA

In sex-stratified analyses in AA, the *G6PD* rs76723693 variant was associated with a decrease in HbA1c of 0.98%-units (95% CI 0.51–1.44) per allele in hemizygous men and 0.46%-units (95% CI 0.26–0.66) in heterozygous women, whereas the *G6PD* rs1050828 variant was associated with a decrease in HbA1c of 0.88%-units (95% CI 0.81–0.95) per allele in hemizygous men and 0.34%-units (95% CI 0.30–0.39) in heterozygous women (Table 2 and

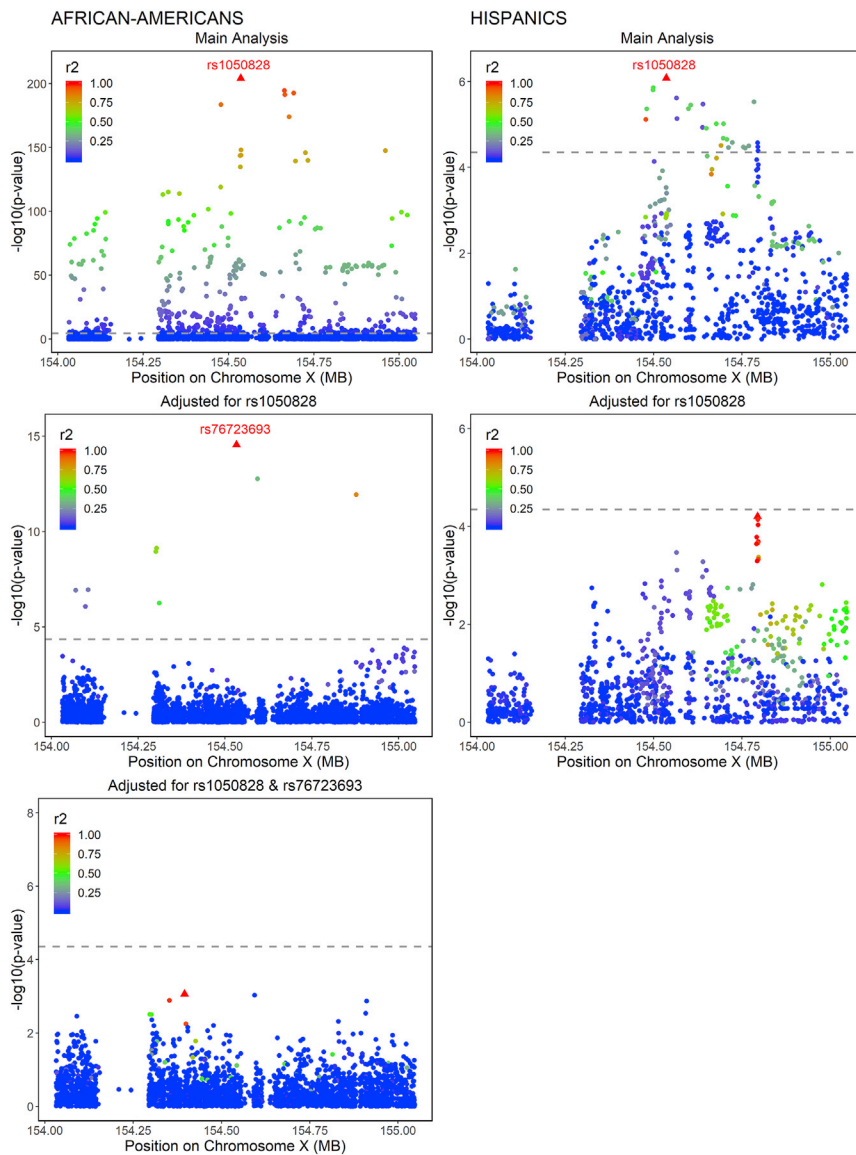


Figure 3. Sequential Conditional Analyses in the *G6PD* Region in Non-Diabetic African-Americans and Hispanics in TOPMed Cohorts

Association plots are displayed separately in African-Americans (left side) and in Hispanics (right side). Single-nucleotide variants are plotted with their p values ($-\log_{10}$ values, y axis) as a function of build 38 genomic position on chromosome X (x axis). The local linkage disequilibrium (LD) structure (r^2) between the top associated single-nucleotide variant (red triangle) and correlated proxies is indicated in color according to a blue to red scale from $r^2 = 0$ to 1 and was calculated separately in non-diabetic African-American and in Hispanic TOPMed cohorts. Significance thresholds to declare an association signal as distinct are indicated by a dashed grey line ($p = 1.7 \times 10^{-5}$ in African-Americans and $p = 4.5 \times 10^{-5}$ in Hispanics, based on the number of single-nucleotide variants in the region with a minor allele count greater than 20).

Figure 4). We detected heterogeneity in effect sizes between males and females for rs1050828 ($P_{\text{het}} = 0.008$) but not for rs76723693 ($P_{\text{het}} = 0.19$). In HA, the *G6PD* rs1050828 variant was associated with a decrease in HbA1c of 0.84%-units (95% CI 0.47–1.22) per allele in hemizygous men and 0.25%-units (95% CI 0.06–0.45) in heterozygous women (Table 3 and Figure 4). Upon adjusting on the SCT variant, rs334, we found that the association of these two SNVs did not attenuate (Table S12).

Population Reclassification of Diabetes Diagnosis Based on *G6PD* Variants

Despite the lower diagnostic sensitivity of HbA1c^{28,29} compared to FG or 2 h glucose in an oral glucose tolerance test, diabetes screening using only HbA1c is not uncommon in clinical practice due to its lower intra-individual variability and the convenience of performing the test in the non-fasting state. To assess the potential public health impact of *G6PD* variants in AA, we designed a hypothetical

scenario, using National Health and Nutrition Examination Survey (NHANES, see URL in the [Web Resources](#) section) 2015–2016, and estimated the number of African Americans and Hispanics whose diagnosis of diabetes would have been missed if they had been screened by a single HbA1c measurement without confirmatory testing by direct glucose measurements. We assumed that the effects of the two *G6PD* variants rs1050828 and rs76723693 were not accounted for, and the standard diagnostic threshold of $\geq 6.5\%$ was uniformly applied to the entire population. We restricted the NHANES analytic sample to adults aged ≥ 18 years and who self-identified as “Non-Hispanic Black” or “Mexican American/Other Hispanic” respectively. We defined the proportions of individuals carrying the *G6PD* variants by their allele frequencies and assumed that the “true” HbA1c values of these individuals were higher by their genetic effect sizes from the association analyses.

In non-Hispanic Black individuals ($n = 1,227$), an estimated 2.32% with HbA1c $< 6.5\%$ -units may be considered to have diabetes upon accounting for the effect size and observed allele frequency of the common *G6PD* variant, rs1050828. An additional 0.13% with HbA1c $< 6.5\%$ -units may be considered to have diabetes upon accounting for the effect of the rare *G6PD* variant, rs76723693. In Mexican American/Other Hispanic individuals ($n = 1,768$), an additional 0.26% with HbA1c $< 6.5\%$ -units may be considered to have diabetes due to the effect of rs1050828. According to the 2016 United States Census

Table 2. Combined and Sex-Stratified Conditional Association Analyses of HbA1c for the Two Distinct Single Nucleotide Variants in the G6PD Region in Non-Diabetic African-Americans in TOPMed Cohorts																		
MarkerID ^a	rsID	Ref/Alt Allele	Pooled Analysis (n = 3,123)				Conditional Analysis on rs1050828				Males (n = 1,269)				Females (n = 1,854)			
			AAF ^b	Beta	SE	p Value	Beta	SE	p Value	Beta	SE	p Value	Beta	SE	p Value	Beta	SE	p Value
X:154533025	rs76723693	A/G	0.005	-0.44	0.07	2.2E-09	-0.50	0.06	2.8E-15	-0.52	0.12	6.8E-6	-0.33	0.10	6.2E-04	0.19		
X:154536002	rs1050828	C/T	0.12	-0.41	0.01	8.4E-205	-	-	-	-0.44	0.02	2.3E-148	-0.37	0.02	2.7E-69	0.008		

^aMarkerID is defined as Chromosome: Position. Positions are indicated on Build 38.

^bAAF: Alternate allele frequency

^cHeterogeneity p Value: test of difference in effect sizes between males and females, accounting for the correlation between males and females statistics

Bureau, approximately 30.14 and 39.33 million adults identified themselves as African American and Hispanic adults, respectively; this suggests that 740,000 African American adults, of whom 40,000 would have diabetes attributed to the rare variant, rs76723693, and 100,000 Hispanic adults with diabetes would remain undiagnosed when screened by a single HbA1c measurement if this genetic information was not taken into account (Tables S15 and S16).

Replication of Results in the UKBB

We selected a total of 5,311 African-ancestry, 398,122 European-ancestry, and 1,331 Asian-ancestry non-diabetic UKBB participants for replication. In the African-ancestry sample, 2,247 participants were males, mean age (SD) was 51.2 (7.8), and mean HbA1c (SD) was 5.5 (0.5). In the European-ancestry sample, 179,055 participants were males, mean age (SD) was 56.6 (8.0), and mean HbA1c (SD) was 5.3 (0.3). In the Asian-ancestry sample, 490 participants were males, mean age (SD) was 52.1 (7.6), and the mean HbA1c (SD) was 5.5 (0.4) (see Supplemental Data).

The four variants of interest (rs334 [SCT], rs1039215 [HBG2], rs76723693, and rs1050828 [G6PD]) had a good quality of imputation (info score > 0.50, Table 4). We observed significant and consistent associations of the two G6PD variants with HbA1c (rs1050828-T, B = -0.38, p < 2.2 × 10⁻¹⁶ and rs76723693-G, B = -0.20, p = 0.008, Table 4). The association of the rare G6PD rs76723693-G variant was even more significant when both G6PD variants were in the model (B = -0.25, p = 5.1 × 10⁻⁵). We observed a high concordance between exome sequence data and imputed genotypes for the rare G6PD rs76723693 SNV. Only seven individuals among 49,741 had a discordance of genotypes between imputed and exome sequence data, using dosage ranges of [0–0.2], [0.9–1.1], and [1.8–2.0] to classify AA, GA, and GG genotypes respectively. The additional G6PD variant with 10 < MAC < 20 in TOPMed, rs5030872, was not present in the UKBB. Thus, GWAS, even with large sample sizes, are limited to genetic variants present in imputation reference panels. We did not detect significant associations of HBG2 and SCT variants with HbA1c in the UKBB. Association results for the TOPMed genome-wide and sub-genome-wide associations in UKBB are provided in Tables 4 and S17. In UKBB African-ancestry participants, in addition to G6PD, we replicated the known HbA1c loci GCK and FN3K. We also found nominal associations with HbA1c (p < 0.05) for rs77974976 in CTTNBP21 (B = -0.12, p = 0.02), rs143745197 in ATP2B3 (B = -0.21, p = 2.4 × 10⁻⁶), and rs189305788 in F8 (B = -0.18 p = 0.01). The SNV rs143745197 was in LD with our main G6PD signal (B = -0.02, p = 0.64 after conditioning on rs1050828) and the SNV rs189305788 was in LD with our additional distinct G6PD signal (B = -0.03, p = 0.83 after conditioning on rs76723693). We did not replicate the sub-genome-wide signal in XPNPEP1 (B = -0.01, p = 0.95). However, the genetic

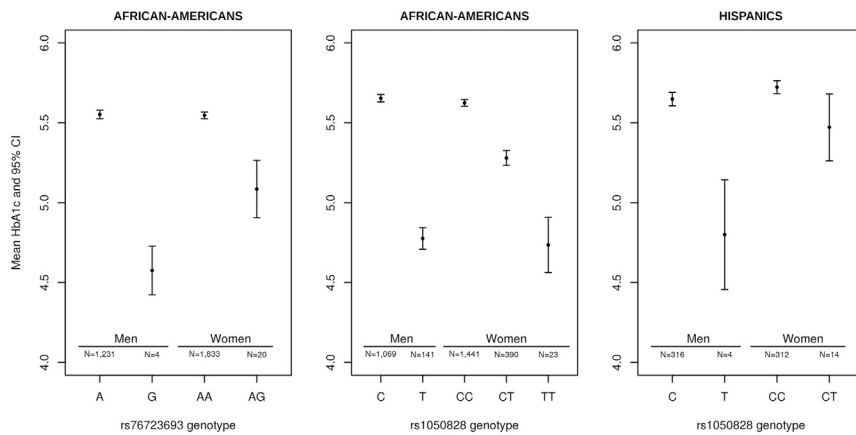


Figure 4. Mean HbA1c Levels According to Genotypes at Both *G6PD* Variants (rs76723693 and rs1050828) and Stratified by Sex

Plots are displayed separately in African-Americans (AA, left and center) and in Hispanics (HA, right). Due to the limited sample size of African-American women carrying two copies of the rare G allele at rs76723693, they are not represented on this plot. In AA, the *G6PD* rs76723693 variant was associated with a decrease in HbA1c of 0.98%-units (95% confidence interval [CI] 0.51–1.44) per allele in hemizygous men and 0.46%-units (95% CI 0.26–0.66) in heterozygous women; whereas the *G6PD* rs1050828 variant was associated with a decrease in HbA1c of 0.88%-units

(95% CI 0.81–0.95) per allele in hemizygous men and 0.34%-units (95% CI 0.30–0.39) in heterozygous women. In HA, the *G6PD* rs1050828 variant was associated with a decrease in HbA1c of 0.84%-units (95% CI 0.47–1.22) per allele in hemizygous men and 0.25%-units (95% CI 0.06–0.45) in heterozygous women.

variant rs551601853 was rarer in the UKBB compared to TOPMed (MAC = 6 in UKBB versus MAC = 20 in TOPMed). In UKBB European-ancestry participants, we replicated the known HbA1c loci: *GCK*, *HK1*, *FN3K*, *G6PC2*, *TMC8*, and *TMPRSS6*. We did not replicate the Asian-specific signal in *EMCN*.

Discussion

Through association analysis and fine-mapping of HbA1c-associated loci in 10,338 individuals from four different ancestries, we demonstrated how deep large-scale WGS can be used to identify common, low frequency, and rare genetic variants that influence nonglycemic variation in HbA1c in multi-ethnic populations. While fewer loci were uncovered in this WGS study compared to array genotyping in larger studies, WGS did enhance our understanding of the allelic heterogeneity at known HbA1c-associated loci. Moreover, the multi-ethnic panel provided more resolution on the range of alleles and haplotype effects within these loci. We confirmed four known regions associated with HbA1c (the glycaemic *GCK*, erythrocytic *HK1* and *G6PD*, and unclassified *FN3K* and/or *FN3KRP* regions) and identified two AA-specific low frequency or rare erythrocytic variants in *G6PD* (rs76723693) and *HBG2* and *HBE1* (rs1039215); these variants, to our knowledge, have not been reported in previous trans-ethnic GWAS meta-analyses of HbA1c. The magnitude and direction of effect of the association of rs76723693 and rs1039215 with HbA1c was similar in the two largest TOPMed AA cohorts (JHS and MESA, Table S18). The association of rs76723693 with HbA1c was replicated in UKBB African-ancestry participants. We detected significant association through the use of a gene-based test when aggregating rare missense variants in *G6PD*; this result indicates that other rare missense variants in *G6PD*, in addition to rs76723693, are associated with HbA1c. We showed that the association of the *HBG2* and *HBE1* variant,

rs1039215, with HbA1c was partially distinct from the known SCT rs334 variant in *HBB* (MIM: 141900), and this suggests that genetic variation at hemoglobin genes other than *HBB* may also influence HbA1c. Individuals carrying both the *G6PD* variant (rs1050828) and the *HBG2* and *HBE1* variant (rs1039215) had even lower HbA1c than did those carrying only one of the two variants. Hemizygous males and homozygous females for the *G6PD* rs1050828-T allele who carried one or more copies of the *HBG2* rs1039215-G allele had a mean HbA1c that was 1.05%-unit lower than the mean HbA1c of individuals who carried none of these alleles.

The HbA1c variant in AA, rs1039215 on chromosome 11, lies in an intron of the hemoglobin subunit gamma 2 gene *HBG2* and the hemoglobin subunit epsilon 1 gene *HBE1*. *HBG2*, in addition to *HBG1*, encodes the gamma chain of hemoglobin, which combines with two alpha chains to form fetal hemoglobin. Fetal hemoglobin is known to interfere with the measurement of HbA1c by some laboratory assays,^{30–32} and so persistence of fetal hemoglobin may be a mechanism through which rs1039215 influences HbA1c measurements. While a small degree of analytic interference with SCT has been reported for the Tosoh 2.2 and G7 assays used by JHS, MESA, and ARIC to measure HbA1c (see NGSP website URL in the [Web Resources](#) section),^{33,34} no interference has been reported for the Bio-Rad Variant II Turbo assay used by UKB³⁵ (Table S2). Thus, assay interference may explain the lack of association of rs334 with HbA1c in UKBB. Alternatively, as our haplotype analysis indicated that the haplotype containing both rs334-A in *HBB* and rs1039215-G in *HBG2* was likely the main driver of this signal in the region, the true causal variant could be another variant within this haplotype that partially explains the reported association of rs334 with HbA1c.

Both variants identified in *G6PD* in AA are missense and pathogenic variants for *G6PD* deficiency (as reported in ClinVar [VCV000037123 and VCV000010388]), an X-linked genetic disorder characterized by a defect in

Table 3. Analysis of HbA1c Associations with rs1050828, X:154536002, Alternate Allele T, in Non-Diabetic African-Americans and Hispanics in TOPMed Cohorts

	African-Americans						Hispanics					
	N	AAF ^a	Beta	SE	p Value	% Of Variance Explained	n	AAF ^a	Beta	SE	p Value	% of Variance Explained
All	3,123	0.12	-0.41	0.01	8.4E-205	0.23	650	0.02	-0.37	0.07	8.3E-07	0.04
Males	1,269	0.12	-0.44	0.02	2.3E-148	0.35	323	0.01	-0.45	0.11	3.5E-05	0.05
Females	1,854	0.12	-0.37	0.02	2.7E-69	0.14	327	0.02	-0.28	0.10	4.5E-03	0.02

^aAAF: alternate allele frequency

glucose-6-phosphate dehydrogenase enzymatic action; this defect causes erythrocytes to break down prematurely. Thus, the mechanism through which these *G6PD* variants lower HbA1c is most likely shortening of the erythrocytic lifespan (Table S19). Assuming random X inactivation, we would expect the difference between hemizygous males and males who do not carry the rs1050828-T variant to be two times larger than the difference between heterozygous females and females who do not carry the rs1050828-T variant; however, in this study, we observed a slightly lower than expected effect in females. We posit that this heterogeneity of genetic effects based on sex may be due to X-linked dosage compensation and not actual sex differences.³⁶ While these *G6PD* variants lower HbA1c in AA, with the same direction of effect as the sickle cell trait variant,⁵ we note that the lowering effect of these genetic variants on HbA1c does not explain the higher mean HbA1c in AA individuals compared to EA individuals with similar glycemia that has been reported in observational studies.^{37,38}

We found that rs1050828, the common *G6PD* variant in AA, was also associated with HbA1c in HA (sample composed of 51% Mexican, 14% Dominican, 14% Puerto Rican, 11% South American, and 10% others) suggesting

that carriers of this variant are likely to be underdiagnosed if a single HbA1c measurement is used to screen two of the largest racial and ethnic minorities in the United States for diabetes. If *G6PD* variants were ignored, we estimate that approximately 840,000 African American and Hispanic adults with diabetes would remain undiagnosed. Because Hispanic populations are ancestrally diverse, and that diversity includes a range of African ancestry (~7% of HA in our study have > 50% AA ancestry and ~25% have > 15% AA ancestry), *G6PD* variants were expected to occur at some lower frequency in HA compared to AA. Given that there is no universal screening for *G6PD* deficiency in North America, asymptomatic carriers are unlikely to know their *G6PD* status.⁷ While misclassification of diabetes status at the population level was largely driven by the common *G6PD* variant rs1050828 and not the rare rs76723693 variant (found in 0.5% of the AA population), in this era of precision medicine, there is a growing need for clinical practice to account for the impact of rare genetic variants with clinically meaningful effects on routinely performed diagnostic tests even if the proportion of carriers in the population is small. We also predict that other *G6PD* deficiency variants, particularly common in African, Mediterranean, and Asian populations,³⁹ will

Table 4. Association Results of the Main TOPMed Signals in the UK Biobank

MarkerID ^a	rsID	Closest Gene	Alternate Allele	Imputation Info Score	African Ancestry				European Ancestry			
					Alternate Allele Frequency	Beta	SE	p Value	Alternate Allele Frequency	Beta	SE	p Value
7:44186502	rs2971670	<i>GCK</i>	T	0.999	0.18	0.06	0.01	2.2E-07	0.18	0.04	0.001	7.8E-233
10:69334748	rs17476364	<i>HK1</i>	C	0.997	0.01	-0.08	0.04	5.7E-02	0.11	-0.12	<1E-04	<1E-250
11:5464062	rs1039215	<i>HBG2</i> and <i>HBE1</i>	G	0.939	0.99	-0.02	0.05	6.0E-01	-	-	-	-
11:5227002	rs334	<i>SCT</i>	A	0.892	0.02	-0.002	0.04	9.6E-01	-	-	-	-
17:82739582	rs113373052	<i>FN3K</i>	T	0.998	0.27	0.03	0.01	8.7E-03	0.31	0.03	0.001	2.2E-214
X:154533025	rs76723693	<i>G6PD</i>	G	0.638	0.004	-0.20	0.08	7.6E-03	-	-	-	-
X:154536002	rs1050828	<i>G6PD</i>	T	0.920	0.16	-0.38	0.01	7.3E-310	-	-	-	-

^aMarkerID is defined as Chromosome:Position. Positions are indicated on Build 38.

impact HbA1c's utility as a diagnostic measure. Until genetic information is universally accessible to adjust diagnostic thresholds of imperfect biomarkers, our findings support current clinical practice guidelines from the American Diabetes Association which recommends the use of both FG and HbA1c in combination for diabetes diagnosis, especially given that the diagnostic level of HbA1c is conservative relative to FG.⁴⁰

Strengths of this investigation include the analysis of extremely high-quality deep sequence data from a large, multi-ancestry sample including five studies and four ancestries to investigate the association of genetic variation across the full allelic spectrum with HbA1c, a diagnostic test that is central to the care of individuals with diabetes. As previous GWAS on HbA1c have included a smaller sample of Hispanic ancestry, this study represents a larger genetic discovery effort for ethnic minorities in the United States. Our study demonstrates that both common and rare genetic variation differ across ancestries and inform the unique genetic architecture of HbA1c. We recognize limitations. We acknowledge that even with a high imputation score, the imputation accuracy of rare variants in the UKBB may not be as optimal as that of common variants. Our hypothetical scenario using NHANES data to assess the population impact of *G6PD* genetics on diabetes screening does not take into account the full complement of clinical and genetic information contributing to nonglycemic variation in HbA1c, some of which may raise, and not lower, HbA1c, thus reducing the risk of under-detection.⁴¹ For instance, structural variants (e.g., copy number variation) were not included in our analyses. Nevertheless, our findings highlight the potential for underdiagnosis in carriers of these variants, which disproportionately affects certain ethnic minorities, if HbA1c is used as the only diagnostic test for diabetes. Our sample sizes, and thus our power, are limited compared to GWAS, particularly for Hispanic and Asian populations. For instance, we did not detect an association signal for rs76723693 in HA because only one individual in our HA population carried a copy of the rare variant. Including larger samples of individuals of non-European ancestries will improve the characterization of rare variants with clinically meaningful effects on HbA1c. We acknowledge that the genome-wide threshold used in this paper may not represent the true number of independent genetic variants (SNVs as well as insertion-deletion variants) tested using our sequence data.

In this study, we identified common and rare genetic variants that cause nonglycemic differences in HbA1c with important clinical and public health implications. Because HbA1c is commonly used in diabetes screening and management worldwide, disregarding the effects of these rare and common genetic variants that tend to occur in high diabetes-risk minority groups could result in delayed diagnosis or under-detection of diabetes in carriers of these variants. To avoid such disparities in care, an assessment of these variants should be considered for incorporation into precision medicine approaches for diabetes diagnosis.

Supplemental Data

Supplemental Data can be found online at <https://doi.org/10.1016/j.ajhg.2019.08.010>.

Acknowledgments

Whole-genome sequencing: Whole-genome sequencing (WGS) for the Trans-Omics in Precision Medicine (TOPMed) program was supported by the National Heart, Lung and Blood Institute (NHLBI). Acknowledgments and funding information for each study are available in the [Supplemental Data](#). Centralized read mapping and genotype calling, along with variant quality metrics and filtering, were provided by the TOPMed Informatics Research Center (3R01HL-117626-02S1). Phenotype harmonization, data management, sample-identity QC, and general study coordination were provided by the TOPMed Data Coordinating Center (3R01HL-120393-02S1). We gratefully acknowledge the studies and participants who provided biological samples and data for TOPMed. The contributions of the investigators of the NHLBI TOPMed Consortium are gratefully acknowledged. Full authorship list is available in the [Supplemental Data](#).

Funding: L.M.R. is funded by NHLBI grant T32 HL129982. A.K.M. is supported by National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grants K01 DK107836 and R03 DK118305. P.S.dV. is supported by American Heart Association grant 18CDA34110116. J.B.M. and the project are supported by NIH NIDDK grants K24 DK080140, U01 DK078616, and U01 DK105554 Opportunity Pool OP6. The Analysis Commons was funded by R01HL131136. H.M.H. was supported by NHLBI training grants T32 HL007055 and T32 HL129982 and American Diabetes Association grant 1-19-PDF-045. J.R.O. is supported by the TOPMed grant U01 HL137181.

Declaration of Interests

The authors declare no competing interests. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

Received: May 14, 2019

Accepted: August 20, 2019

Published: September 26, 2019

Web Resources

Analysis Commons, <http://analysiscommons.com>

Database of Genotypes and Phenotypes (dbGaP), <https://www.ncbi.nlm.nih.gov/gap/>

GENetic ESTimation and Inference in Structured samples (GENESIS), <http://bioconductor.org/packages/release/bioc/html/GENESIS.html>

HbA1c Assay Interferences information on the National Glycohemoglobin Standardization Program (NGSP) website, <http://www.ngsp.org/interf.asp>

Mixed Model Analysis for Pedigrees and Populations (MMAP), <https://mmap.github.io/>

National Health and Nutrition Examination Survey (NHANES): <https://www.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015>

NHLBI TOPMed Consortium, <https://www.nhlbiwgs.org/topmed-banner-authorship>

Omics Analysis, Search and Information System (OASIS), <https://omicsoasis.github.io>

Online Mendelian Inheritance in Man (OMIM), <https://www.omim.org>

PLINK, <http://zzz.bwh.harvard.edu/plink/>

Scalable and Accurate Implementation of Generalized mixed model (SAIGE), <https://github.com/weizhouUMICH/SAIGE>

SmartPCA, <https://www.hsph.harvard.edu/alkes-price/software>

TOPMed laboratory methods, data processing, and quality control details, <https://www.nhlbiwgs.org/methods>

TOPMed Pipeline, https://github.com/UW-GAC/analysis_pipeline

TOPMed website, <https://www.nhlbiwgs.org>

Trans-Omics for Precision Medicine (TOPMed) Consortium full authorship list, <https://www.nhlbiwgs.org/topmed-banner-authorship>

Type 2 Diabetes Knowledge Portal, <http://www.type2diabetesgenetics.org>

UK Biobank, <https://www.ukbiobank.ac.uk/>

References

- Mortensen, H.B., and Christophersen, C. (1983). Glucosylation of human haemoglobin a in red blood cells studied in vitro. Kinetics of the formation and dissociation of haemoglobin A1c. *Clin. Chim. Acta* 134, 317–326.
- Leong, A., and Meigs, J.B. (2015). Type 2 diabetes prevention: Implications of hemoglobin A1c genetics. *Rev. Diabet. Stud.* 12, 351–362.
- Wheeler, E., Leong, A., Liu, C.T., Hivert, M.F., Strawbridge, R.J., Podmore, C., Li, M., Yao, J., Sim, X., Hong, J., et al.; EPIC-CVD Consortium; EPIC-InterAct Consortium; and Lifelines Cohort Study (2017). Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. *PLoS Med.* 14, e1002383.
- Frazer, K.A., Ballinger, D.G., Cox, D.R., Hinds, D.A., Stuve, L.L., Gibbs, R.A., Belmont, J.W., Boudreau, A., Hardenbol, P., Leal, S.M., et al.; International HapMap Consortium (2007). A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449, 851–861.
- Lacy, M.E., Wellenius, G.A., Sumner, A.E., Correa, A., Carnethon, M.R., Liem, R.I., Wilson, J.G., Sacks, D.B., Jacobs, D.R., Jr., Carson, A.P., et al. (2017). Association of sickle cell trait with Hemoglobin A1c in African Americans. *JAMA* 317, 507–515.
- Strickland, S.W., Campbell, S.T., Little, R.R., Bruns, D.E., and Bazydlo, L.A.L. (2018). Recognition of rare hemoglobin variants by hemoglobin A_{1c} measurement procedures. *Clin. Chim. Acta* 476, 67–74.
- Leong, A. (2007). Is there a need for neonatal screening of glucose-6-phosphate dehydrogenase deficiency in Canada? *McGill J. Med.* 10, 31–34.
- Motulsky, A.G., and Stamatoyannopoulos, G. (1966). Clinical implications of glucose-6-phosphate dehydrogenase deficiency. *Ann. Intern. Med.* 65, 1329–1334.
- Paterson, A.D. (2017). HbA1c for type 2 diabetes diagnosis in Africans and African Americans: Personalized medicine NOW!. *PLoS Med.* 14, e1002384.
- Little, R.R., Rohlfing, C.L., Sacks, D.B.; and National Glycohemoglobin Standardization Program (NGSP) Steering Committee (2011). Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care. *Clin. Chem.* 57, 205–214.
- Regier, A.A., Farjoun, Y., Larson, D.E., Krasheninina, O., Kang, H.M., Howrigan, D.P., Chen, B.J., Kher, M., Banks, E., Ames, D.C., et al. (2018). Functional equivalence of genome sequencing analysis pipelines enables harmonized variant calling across human genetics projects. *Nat. Commun.* 9, 4038.
- Zheng, X., Gogarten, S.M., Lawrence, M., Stilp, A., Conomos, M.P., Weir, B.S., Laurie, C., and Levine, D. (2017). SeqArray—a storage-efficient high-performance data format for WGS variant calls. *Bioinformatics* 33, 2251–2257.
- Brody, J.A., Morrison, A.C., Bis, J.C., O’Connell, J.R., Brown, M.R., Huffman, J.E., Ames, D.C., Carroll, A., Conomos, M.P., Gabriel, S., et al.; NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium; Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium; TOPMed Hematology and Hemostasis Working Group; and CHARGE Analysis and Bioinformatics Working Group (2017). Analysis commons, a team approach to discovery in a big-data environment for genetic epidemiology. *Nat. Genet.* 49, 1560–1563.
- Amin, N., van Duijn, C.M., and Aulchenko, Y.S. (2007). A genomic background based method for association analysis in related individuals. *PLoS ONE* 2, e1274.
- Leutenegger, A.L., Prum, B., Génin, E., Verny, C., Lemainque, A., Clerget-Darpoux, F., and Thompson, E.A. (2003). Estimation of the inbreeding coefficient through use of genomic data. *Am. J. Hum. Genet.* 73, 516–523.
- Kanai, M., Tanaka, T., and Okada, Y. (2016). Empirical estimation of genome-wide significance thresholds based on the 1000 Genomes Project data set. *J. Hum. Genet.* 61, 861–866.
- Xu, C., Tachmazidou, I., Walter, K., Ciampi, A., Zeggini, E., Greenwood, C.M.; and UK10K Consortium (2014). Estimating genome-wide significance for whole-genome sequencing studies. *Genet. Epidemiol.* 38, 281–290.
- Astle, W.J., Elding, H., Jiang, T., Allen, D., Ruklisa, D., Mann, A.L., Mead, D., Bouman, H., Riveros-Mckay, F., Kostadima, M.A., et al. (2016). The Allelic Landscape of Human Blood Cell Trait Variation and Links to Common Complex Disease. *Cell* 167, 1415–1429.e19.
- Hodonsky, C.J., Jain, D., Schick, U.M., Morrison, J.V., Brown, L., McHugh, C.P., Schurmann, C., Chen, D.D., Liu, Y.M., Auer, P.L., et al. (2017). Genome-wide association study of red blood cell traits in Hispanics/Latinos: The Hispanic Community Health Study/Study of Latinos. *PLoS Genet.* 13, e1006760.
- Chen, Z., Tang, H., Qayyum, R., Schick, U.M., Nalls, M.A., Handsaker, R., Li, J., Lu, Y., Yanek, L.R., Keating, B., et al.; BioBank Japan Project; and CHARGE Consortium (2013). Genome-wide association analysis of red blood cell traits in African Americans: the COGENT Network. *Hum. Mol. Genet.* 22, 2529–2538.
- Lo, K.S., Wilson, J.G., Lange, L.A., Folsom, A.R., Galarneau, G., Ganesh, S.K., Grant, S.F., Keating, B.J., McCarroll, S.A., Mohler, E.R., 3rd., et al. (2011). Genetic association analysis highlights new loci that modulate hematological trait variation in Caucasians and African Americans. *Hum. Genet.* 129, 307–317.
- Chami, N., Chen, M.H., Slater, A.J., Eicher, J.D., Evangelou, E., Tajuddin, S.M., Love-Gregory, L., Kacprowski, T., Schick, U.M., Nomura, A., et al. (2016). Exome Genotyping Identifies

- Pleiotropic Variants Associated with Red Blood Cell Traits. *Am. J. Hum. Genet.* *99*, 8–21.
23. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* *26*, 2190–2191.
 24. Liu, X., White, S., Peng, B., Johnson, A.D., Brody, J.A., Li, A.H., Huang, Z., Carroll, A., Wei, P., Gibbs, R., et al. (2016). WGS: An annotation pipeline for human genome sequencing studies. *J. Med. Genet.* *53*, 111–112.
 25. Bycroft, C., Freeman, C., Petkova, D., Band, G., Elliott, L.T., Sharp, K., Motyer, A., Vukcevic, D., Delaneau, O., O'Connell, J., et al. (2018). The UK Biobank resource with deep phenotyping and genomic data. *Nature* *562*, 203–209.
 26. Zhou, W., Nielsen, J.B., Fritsche, L.G., Dey, R., Gabrielsen, M.E., Wolford, B.N., LeFaive, J., VandeHaar, P., Gagliano, S.A., Gifford, A., et al. (2018). Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat. Genet.* *50*, 1335–1341.
 27. Shriner, D., and Rotimi, C.N. (2018). Whole-genome-sequence-based haplotypes reveal single origin of the sickle allele during the Holocene wet phase. *Am. J. Hum. Genet.* *102*, 547–556.
 28. Cowie, C.C., Rust, K.F., Byrd-Holt, D.D., Gregg, E.W., Ford, E.S., Geiss, L.S., Bainbridge, K.E., and Fradkin, J.E. (2010). Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988–2006. *Diabetes Care* *33*, 562–568.
 29. Selvin, E., Steffes, M.W., Gregg, E., Brancati, F.L., and Coresh, J. (2011). Performance of A1C for the classification and prediction of diabetes. *Diabetes Care* *34*, 84–89.
 30. Little, R.R., and Roberts, W.L. (2009). A review of variant hemoglobins interfering with hemoglobin A1c measurement. *J. Diabetes Sci. Technol.* *3*, 446–451.
 31. Rohlfing, C.L., Connolly, S.M., England, J.D., Hanson, S.E., Moellering, C.M., Bachelder, J.R., and Little, R.R. (2008). The effect of elevated fetal hemoglobin on hemoglobin A1c results: five common hemoglobin A1c methods compared with the IFCC reference method. *Am. J. Clin. Pathol.* *129*, 811–814.
 32. Sarnowski, C., and Hivert, M.F. (2018). Impact of genetic determinants of HbA1c on Type 2 diabetes risk and diagnosis. *curr. diab. rep.* *18*, 52.
 33. Roberts, W.L., Safar-Pour, S., De, B.K., Rohlfing, C.L., Weykamp, C.W., and Little, R.R. (2005). Effects of hemoglobin C and S traits on glycohemoglobin measurements by eleven methods. *Clin. Chem.* *51*, 776–778.
 34. Rohlfing, C., Hanson, S., and Little, R.R. (2017). Measurement of hemoglobin A1c in patients with sickle cell trait. *JAMA* *317*, 2237.
 35. Mongia, S.K., Little, R.R., Rohlfing, C.L., Hanson, S., Roberts, R.F., Owen, W.E., D'Costa, M.A., Reyes, C.A., Luzzi, V.I., and Roberts, W.L. (2008). Effects of hemoglobin C and S traits on the results of 14 commercial glycated hemoglobin assays. *Am. J. Clin. Pathol.* *130*, 136–140.
 36. Sidorenko, J., Kassam, I., Kemper, K., Zeng, J., Lloyd-Jones, L., Montgomery, G.W., Gibson, G., Metspalu, A., Esko, T., Yang, J., et al. (2018). The effect of X-linked dosage compensation on complex trait variation. *bioRxiv*.
 37. Bergenstal, R.M., Gal, R.L., Connor, C.G., Gubitosi-Klug, R., Kruger, D., Olson, B.A., Willi, S.M., Aleppo, G., Weinstock, R.S., Wood, J., et al.; T1D Exchange Racial Differences Study Group (2017). Racial differences in the relationship of glucose concentrations and Hemoglobin A1c levels. *Ann. Intern. Med.* *167*, 95–102.
 38. Ziemer, D.C., Kolm, P., Weintraub, W.S., Vaccarino, V., Rhee, M.K., Twombly, J.G., Narayan, K.M., Koch, D.D., and Phillips, L.S. (2010). Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. *Ann. Intern. Med.* *152*, 770–777.
 39. Gómez-Manzo, S., Marcial-Quino, J., Vanoye-Carlo, A., Serrano-Posada, H., Ortega-Cuellar, D., González-Valdez, A., Castillo-Rodríguez, R.A., Hernández-Ochoa, B., Sierra-Palacios, E., Rodríguez-Bustamante, E., and Arreguin-Espinosa, R. (2016). Glucose-6-Phosphate Dehydrogenase: update and analysis of new mutations around the world. *Int. J. Mol. Sci.* *17*, E2069. <https://doi.org/10.3390/ijms17122069>.
 40. American Diabetes Association (2019). 2. Classification and diagnosis of diabetes: *standards of medical care in diabetes-2019*. *Diabetes Care* *42* (Suppl 1), S13–S28.
 41. Jun, G., Sedlazeck, F.J., Chen, H., Yu, B., Qi, Q., Krasheninina, O., Carroll, A., Liu, X., Mansfield, A., Zarate, S., et al. (2018). PgmNr 3186/W: Identification of novel structural variations affecting common and complex disease risks with >16,000 whole genome sequences from ARIC and HCHS/SOL. <https://www.ashg.org/2018meeting/pdf/ASHG-2018-poster-abstracts.pdf>

Supplemental Data

Impact of Rare and Common Genetic Variants on Diabetes

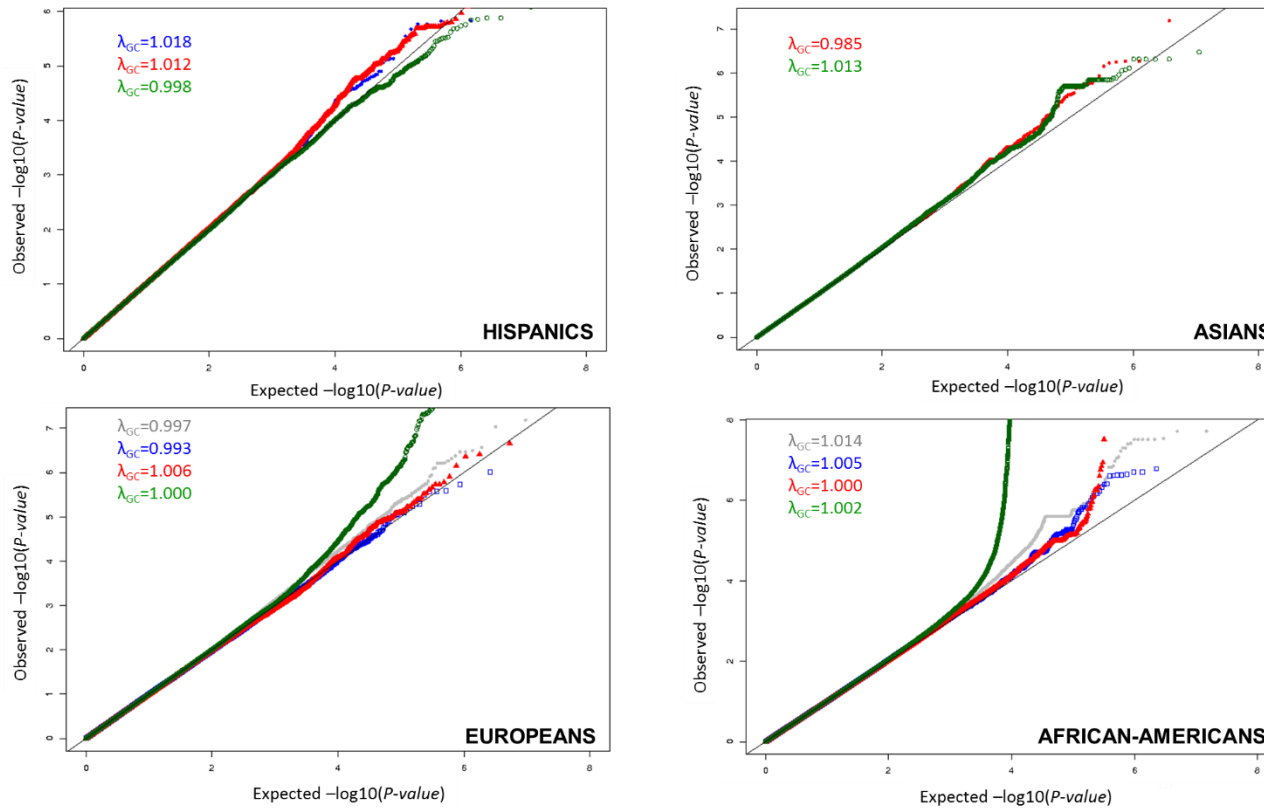
Diagnosis by Hemoglobin A1c in Multi-Ancestry Cohorts:

The Trans-Omics for Precision Medicine Program

Chloé Sarnowski, Aaron Leong, Laura M. Raffield, Peitao Wu, Paul S. de Vries, Daniel DiCorpo, Xiuqing Guo, Huichun Xu, Yongmei Liu, Xiuwen Zheng, Yao Hu, Jennifer A. Brody, Mark O. Goodarzi, Bertha A. Hidalgo, Heather M. Highland, Deepti Jain, Ching-Ti Liu, Rakhi P. Naik, Jeffrey R. O'Connell, James A. Perry, Bianca C. Porneala, Elizabeth Selvin, Jennifer Wessel, Bruce M. Psaty, Joanne E. Curran, Juan M. Peralta, John Blangero, Charles Kooperberg, Rasika Mathias, Andrew D. Johnson, Alexander P. Reiner, Braxton D. Mitchell, L. Adrienne Cupples, Ramachandran S. Vasan, Adolfo Correa, Alanna C. Morrison, Eric Boerwinkle, Jerome I. Rotter, Stephen S. Rich, Alisa K. Manning, Josée Dupuis, James B. Meigs, TOPMed Diabetes Working Group, TOPMed Hematology Working Group, TOPMed Hemostasis Working Group, and National Heart, Lung, and Blood Institute TOPMed Consortium

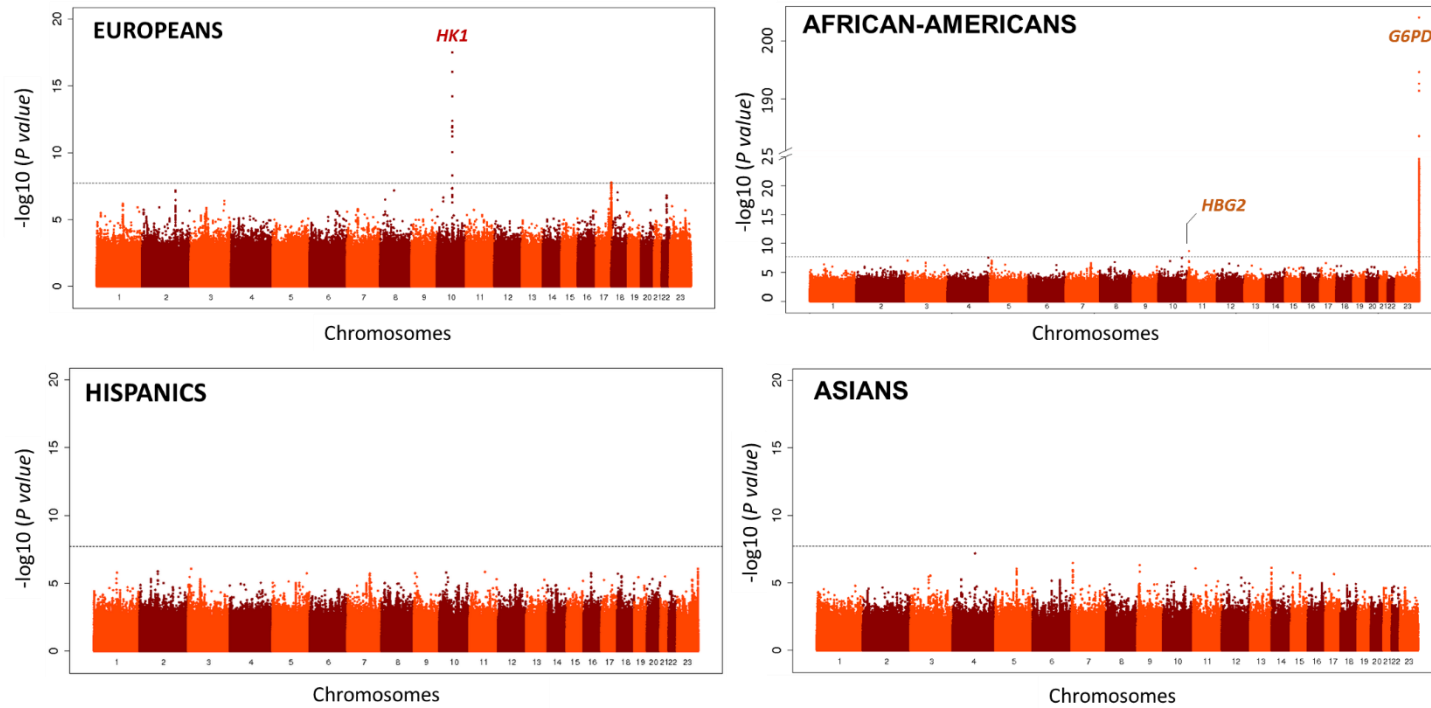
Supplementary Figures

Figure S1: Quantile-Quantile (QQ)-plots by ancestry of the association analysis of HbA1c in non-diabetic individuals in TOPMed cohorts stratified on minor allele frequency (MAF)



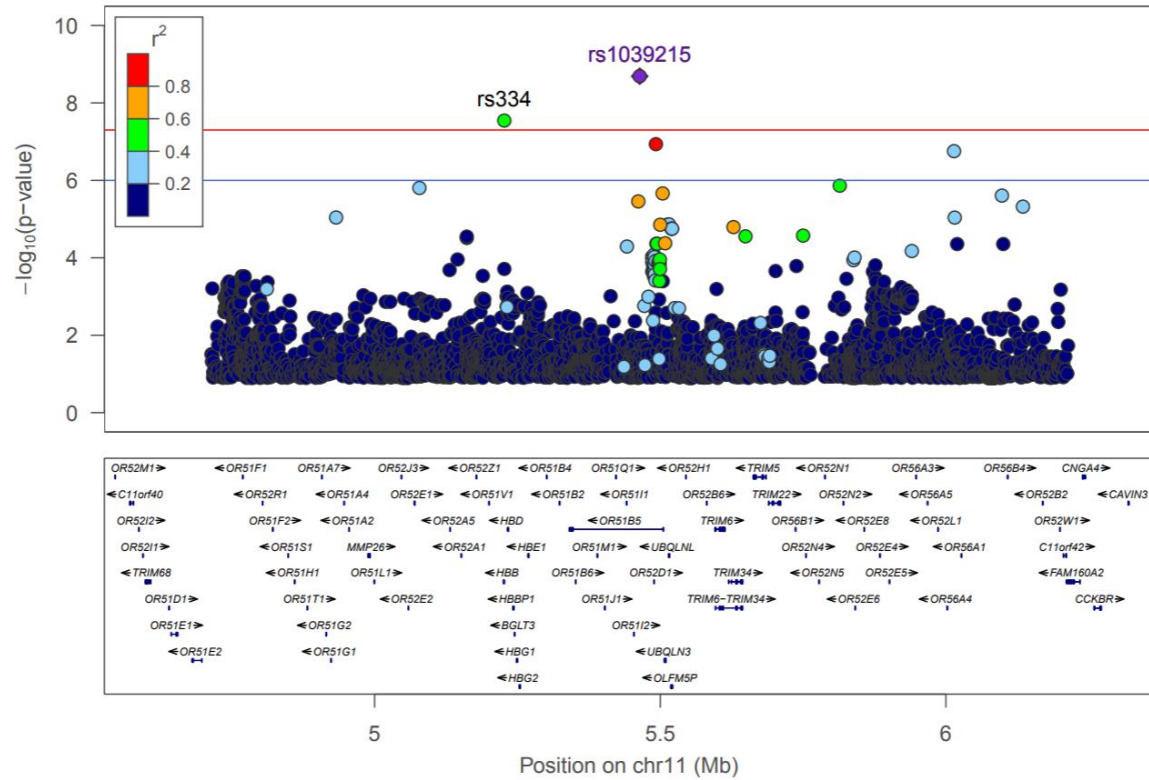
The dots represent the distribution of observed ordered $-\log_{10}(P\text{-values})$ against the theoretical model distribution of expected ordered $-\log_{10}(P\text{-values})$. The solid black line represents the theoretical model distribution of expected $-\log_{10}(P\text{-values})$ under the null distribution. The genomic inflation factor (λ_{GC}) is defined as the ratio of the median of the empirically observed distribution of the test statistic to the expected median, thus quantifying the extent of the inflation and the excess false positive rate. Association results were stratified by minor allele frequency (MAF) of single nucleotide variants: $MAF \geq 5\%$ are indicated in green; $5\% > MAF \geq 1\%$ are indicated in red; $1\% > MAF \geq 0.5\%$ are indicated in blue; $0.5\% > MAF \geq 0.1\%$ are indicated in grey.

Figure S2: Manhattan-plots by ancestry of the association analysis of HbA1c in non-diabetic individuals in TOPMed cohorts.



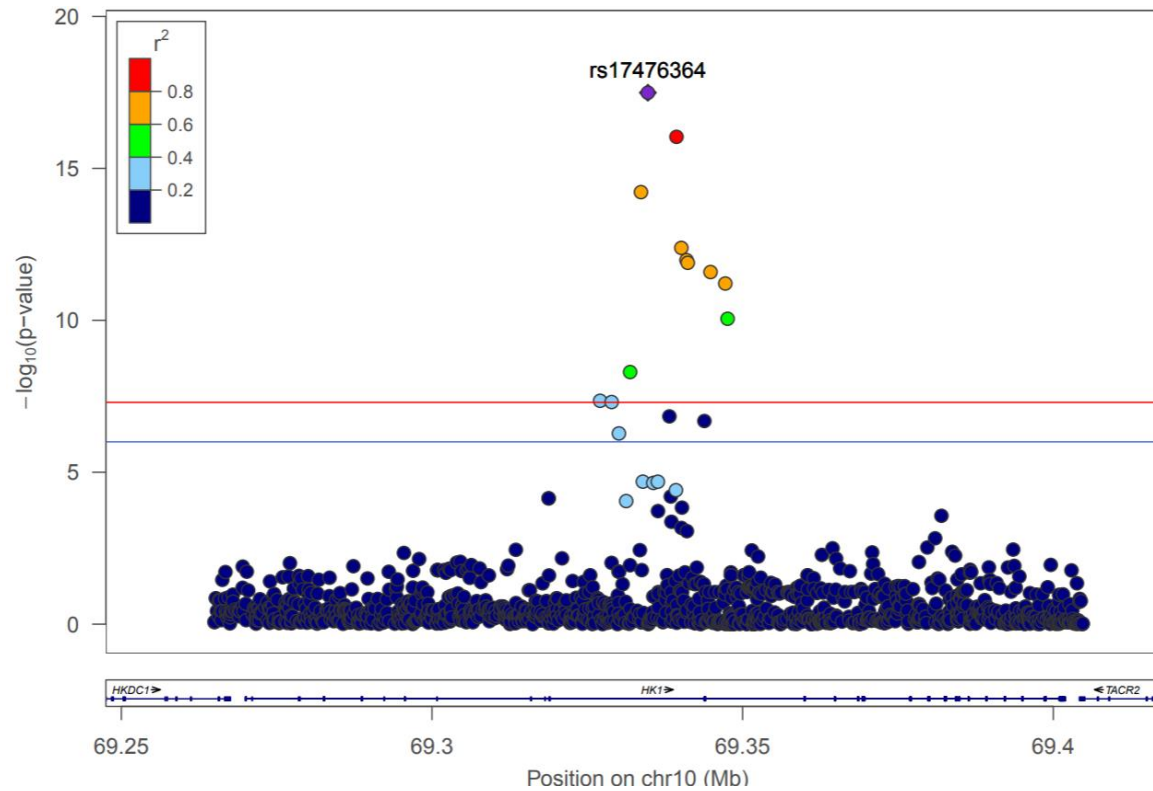
The $-\log_{10}(P\text{-value})$ for each single nucleotide variant on the y-axis is plotted against the build 38 genomic position on the x-axis (chromosomal coordinate). The dashed horizontal line indicates the genome-wide significance threshold of $P = 2 \times 10^{-8}$. The y-axis was truncated for ease of interpretation.

Figure S3: Regional HbA1c association plot in the *HBG2/HBE1* region (+/- 750kb around *HBG2*).



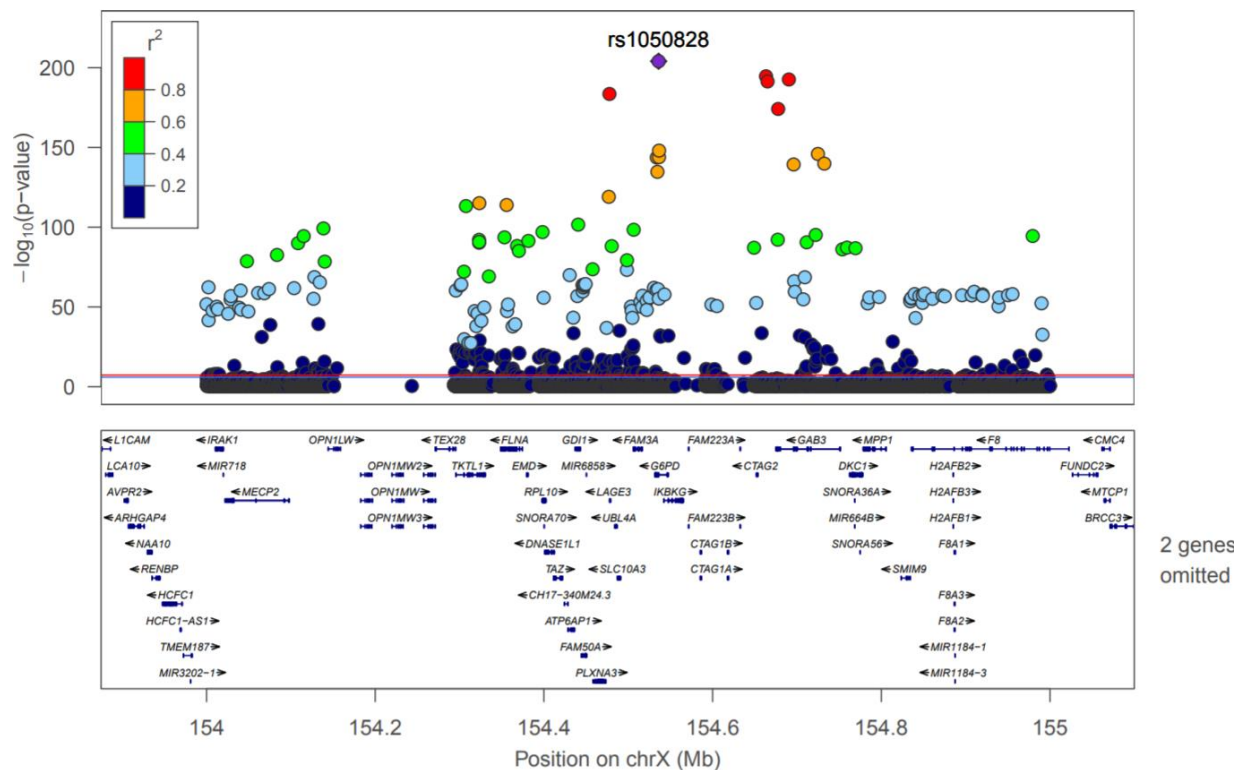
Single nucleotide variants are plotted with their P-values ($-\log_{10}$ values, left y-axis) as a function of build 38 genomic position on chromosome 11 (x-axis). Estimated recombination rates (right y-axis) are plotted to reflect the local linkage disequilibrium (LD) structure around the top associated single nucleotide variant rs1039215 (purple diamond) and correlated proxies (according to a blue to red scale from $r^2=0$ to 1). LD was calculated in non-diabetic African ancestry TOPMed cohorts.

Figure S4: Regional HbA1c association plot in the *HK1* region (± 70 kb around *HK1*).



Single nucleotide variants are plotted with their P-values ($-\log_{10}$ values, left y-axis) as a function of build 38 genomic position on chromosome 10 (x-axis). Estimated recombination rates (right y-axis) are plotted to reflect the local linkage disequilibrium (LD) structure around the top associated single nucleotide variant rs17476364 (purple diamond) and correlated proxies (according to a blue to red scale from $r^2=0$ to 1). LD was calculated in non-diabetic European ancestry TOPMed cohorts.

Figure S5: Regional HbA1c association plot in the *G6PD* region (± 500 kb around *G6PD*).



Single nucleotide variants are plotted with their P-values ($-\log_{10}$ values, left y-axis) as a function of build 38 genomic position on chromosome X (x-axis). Estimated recombination rates (right y-axis) are plotted to reflect the local linkage disequilibrium (LD) structure around the top associated single nucleotide variant rs1050828 (purple diamond) and correlated proxies (according to a blue to red scale from $r^2=0$ to 1). LD was calculated in non-diabetic African ancestry TOPMed cohorts.

Table S1: Counts of non-diabetic individuals by TOPMed cohorts and ancestry groups included in HbA1c association analyses

Cohort	Ancestry				Totals
	European	African-American	Hispanic	Asian	
Amish	151	--	--	--	151
Atherosclerosis Risk in Communities Study (ARIC)	2,315	100	--	--	2,415
Framingham Heart Study (FHS)	2,236	--	--	--	2,236
Jackson Heart Study (JHS)	--	2,356	--	--	2,356
Multi-Ethnic Study of Atherosclerosis (MESA)	1,456	667	650	407	3,180
Totals	6,158	3,123	650	407	10,338

Table S2: Assays used to measure HbA1c by TOPMed cohorts included in HbA1c association analyses and in UK Biobank

Cohort	Assay	Interference ^a	
		HbS (SCT)	Elevated HbF
Amish	Quest Diagnostics using Immunoturbidimetry (Roche Cobas Integra)	Higher	?
Atherosclerosis Risk in Communities Study (ARIC)	2003-04: Tosoh 2.2 Plus HPLC instrument	Slightly lower*	?
	2007-08: Tosoh G7 instrument	Slightly lower*	Yes if >30%
Framingham Heart Study (FHS)	Roche Hitachi 911, Roche Diagnostics, HbA1c - turbidimetric immunoassay	No**	?
Jackson Heart Study (JHS)	Tosoh 2.2 HPLC system (Tosoh Corporation, Tokyo, Japan) at University of Minnesota	Slightly lower*	?
Multi-Ethnic Study of Atherosclerosis (MESA)	Exam 2: Tosoh 2.2 Plus HPLC instrument	Slightly lower*	?
	Exam 5: Tosoh G7 instrument	Slightly lower*	Yes if >30%
UK Biobank	Bio-Rad Variant II Turbo	No**	Yes if >5%

^a Slightly lower*: Some assay inference has been reported with HbS but not $\geq \pm 7\%$ at 6 or 9% HbA1c; No**: Assay inference has not been reported to be $\geq \pm 7\%$ at 6 or 9% HbA1c. ?: Not reported

Table S3: Number of TOPMed individuals, per study, included in the TOPMed red blood cell traits association analyses

Cohort	HB ^a	HCT ^b	MCH ^c	MCHC ^d	MCV ^e	RBC ^f	RDW ^g
Amish	1,021	1,021	1,021	1,021	1,021	1,021	0
Atherosclerosis Risk in Communities Study (ARIC)	3,475	3,475	1,778	1,779	3,315	1,778	1,073
Cardiovascular Health Study (CHS)	69	69	0	0	0	0	0
Framingham Heart Study (FHS)	2,826	2,826	2,826	2,826	2,826	2,826	2,080
Genetic Study of Atherosclerosis Risk (GeneSTAR)	1,585	1,585	1,506	1,506	1,506	1,506	0
Jackson Heart Study (JHS)	2,981	2,981	2,675	2,675	2,674	2,675	2,674
Multi-Ethnic Study of Atherosclerosis (MESA)	2,240	2,240	2,240	2,240	2,240	2,240	0
San Antonio Family Study (SAFS)	971	971	968	968	965	970	0
Women's Health Initiative (WHI)	9,912	9,909	1,286	1,286	1,286	1,286	1,285
Total	25,080	25,077	14,300	14,301	15,833	14,302	7,112

^aHB: Hemoglobin

^bMCV: Mean Corpuscular Volume

^cMCH: Mean Corpuscular Hemoglobin

^dMCHC: Mean Corpuscular Hemoglobin Concentration

^eHCT: Hematocrit

^fRBC: Red Blood Cell count

^gRDW: Red cell Distribution Width

Table S4: Characteristics of non-diabetic individuals by TOPMed cohorts and ancestry groups included in HbA1c association analyses

Cohort	N	Men, N (%)	Age mean years (SD)	HbA1c mean % (SD)	Fasting Glucose (FG) mean mmol/L (SD)	Hemoglobin (Hb) mean mg/L (SD)
Amish European ancestry	151	69 (45.7)	45.1 (15.8)	4.9 (0.4)	5.0 (0.5)	--
ARIC European ancestry	2,315	1,117 (48.3)	57.5 (5.6)	5.4 (0.4)	6.4 (0.6)	14.0 (1.3)
ARIC African ancestry	100	41 (41.0)	56.8 (5.4)	5.6 (0.4)	6.3 (0.5)	13.1 (1.4)
FHS European ancestry Offspring Cohort	1,211	522 (43.1)	71.7 (8.0)	5.6 (0.3)	5.5 (0.5)	13.7 (1.2)
FHS European ancestry Third Generation	1,025	473 (46.2)	46.1 (9.0)	5.4 (0.3)	5.2 (0.5)	14.2 (1.2)
JHS African ancestry	2,356	915 (38.8)	54.0 (13.0)	5.5 (0.5)	5.0 (0.5)	13.2 (1.5)
MESA European ancestry	1,456	703 (48.3)	69.9 (9.5)	5.5 (0.3)	5.1 (0.5)	--
MESA African ancestry	667	313 (46.9)	68.6 (9.6)	5.7 (0.4)	5.1 (0.5)	--
MESA Hispanic ancestry	650	323 (49.7)	68.2 (9.7)	5.7 (0.4)	5.3 (0.5)	--
MESA Asian ancestry	407	206 (50.6)	68.4 (9.3)	5.7 (0.4)	5.2 (0.5)	--

Table S5: Most associated single nucleotide variants in the regions detected at the sub-genome-wide level ($P < 5 \times 10^{-7}$) by ancestry and in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts

MarkerID ^a	rsID	Gene	Ref/Alt Allele	Meta-analysis			Europeans (N=6,158)				African-Americans (N=3,123)			
				Beta	SE	<i>P value</i>	AAF ^b	Beta	SE	<i>P value</i>	AAF ^b	Beta	SE	<i>P value</i>
1:69561493	rs546782425	<i>LRRC7</i>	G/A	-0.37	0.07	4.10E-07	--	--	--	--	0.01	-0.37	0.07	4.10E-07
2:168900844	rs1402837	<i>G6PC2</i>	C/T	0.03	0.01	1.40E-07	0.23	0.04	0.01	6.80E-08	0.29	0.01	0.01	0.29
3:5940489	rs541283713	<i>EDEM1</i>	G/T	-0.48	0.09	8.90E-08	--	--	--	--	0.004	-0.48	0.09	8.90E-08
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	-0.03	0.01	8.90E-08	0.26	-0.03	0.01	1.40E-06	0.06	-0.03	0.02	0.18
4:100766435	rs184930483	<i>EMCN</i>	T/C	-0.33	0.06	6.50E-08	--	--	--	--	--	--	--	--
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-0.32	0.06	4.70E-07	--	--	--	--	0.01	-0.32	0.06	4.70E-07
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	-0.35	0.07	2.40E-07	--	--	--	--	0.01	-0.35	0.07	2.40E-07
7:117730990		<i>CTTNBP2</i>	TG/T	-0.35	0.07	2.40E-07	--	--	--	--	0.01	-0.35	0.07	2.40E-07
7:117734487	rs115069847	<i>CTTNBP2</i>	A/G	-0.34	0.07	3.90E-07	--	--	--	--	0.01	-0.34	0.07	3.90E-07
8:57797437	rs182571425	<i>FAM110B</i>	T/G	-0.35	0.06	6.60E-08	0.002	-0.35	0.06	6.60E-08	--	--	--	--
8:59954108	rs148165085	<i>CA8</i>	C/G	-0.40	0.08	1.60E-07	--	--	--	--	0.01	-0.40	0.08	1.60E-07
10:53414779	rs185736000	<i>PCDH15</i>	G/A	-0.48	0.09	1.10E-07	--	--	--	--	0.004	-0.48	0.09	1.10E-07
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.55	0.10	3.10E-08	--	--	--	--	0.003	-0.55	0.10	3.1E-08
11:5078417	rs77362408	<i>OR52E2</i>	C/G	-0.15	0.03	3.10E-07	--	--	--	--	0.04	-0.14	0.03	1.60E-06
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-0.20	0.04	1.20E-07	--	--	--	--	0.02	-0.20	0.04	1.20E-07
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-0.19	0.04	1.80E-07	--	--	--	--	0.03	-0.19	0.04	1.80E-07
17:32533283	rs140882821	<i>MYO1D</i>	G/A	-0.19	0.04	2.80E-07	--	--	--	--	0.02	-0.19	0.04	2.50E-07
17:78138386	rs144120533	<i>TMC8</i>	C/T	-0.26	0.05	2.70E-07	0.003	-0.26	0.05	2.70E-07	--	--	--	--
18:2770634		<i>SMCHD1</i>	G/GT	0.17	0.03	1.40E-07	0.001	0.16	0.11	0.14	0.03	0.17	0.03	8.40E-07
18:34585879	rs187431535	<i>DTNA</i>	T/A	0.25	0.05	9.20E-08	0.004	0.25	0.05	9.20E-08	--	--	--	--
18:34712254	rs183229631	<i>DTNA</i>	G/A	0.25	0.05	3.40E-07	0.003	0.25	0.05	3.40E-07	--	--	--	--
21:37519947	rs11701836	<i>DYRK1A</i>	A/G	-0.03	0.01	4.40E-07	0.26	-0.02	0.01	1.70E-03	0.32	-0.05	0.01	1.10E-04
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	0.03	0.01	1.10E-07	0.44	0.03	0.01	1.60E-07	0.16	0.01	0.02	0.53
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	-0.31	0.06	2.30E-07	--	--	--	--	0.01	-0.31	0.06	2.30E-07
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	-0.23	0.04	1.40E-07	--	--	--	--	0.01	-0.23	0.04	1.40E-07
X:154877653	rs189305788	<i>F8</i>	C/A	-0.40	0.08	2.40E-07	--	--	--	--	0.01	-0.40	0.08	2.40E-07

Table S5 (continued):

				Meta-analysis			Hispanics (N=650)				Asians (N=407)			
MarkerID ^a	rsID	Gene	Ref/Alt Allele	Beta	SE	<i>P value</i>	AAF ^b	Beta	SE	<i>P value</i>	AAF ^b	Beta	SE	<i>P value</i>
1:69561493	rs546782425	<i>LRRC7</i>	G/A	-0.37	0.07	4.10E-07	--	--	--	--	--	--	--	--
2:168900844	rs1402837	<i>G6PC2</i>	C/T	0.03	0.01	1.40E-07	0.22	0.02	0.03	0.45	0.38	0.01	0.03	0.63
3:5940489	rs541283713	<i>EDEM1</i>	G/T	-0.48	0.09	8.90E-08	--	--	--	--	--	--	--	--
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	-0.03	0.01	8.90E-08	0.13	-0.06	0.03	0.08	0.01	-0.24	0.12	0.04
4:100766435	rs184930483	<i>EMCN</i>	T/C	-0.33	0.06	6.50E-08	--	--	--	--	0.04	-0.33	0.06	6.50E-08
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-0.32	0.06	4.70E-07	--	--	--	--	--	--	--	--
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	-0.35	0.07	2.40E-07	--	--	--	--	--	--	--	--
7:117730990	--	<i>CTTNBP2</i>	TG/T	-0.35	0.07	2.40E-07	--	--	--	--	--	--	--	--
7:117734487	rs115069847	<i>CTTNBP2</i>	A/G	-0.34	0.07	3.90E-07	--	--	--	--	--	--	--	--
8:57797437	rs182571425	<i>FAM110B</i>	T/G	-0.35	0.06	6.60E-08	--	--	--	--	--	--	--	--
8:59954108	rs148165085	<i>CA8</i>	C/G	-0.40	0.08	1.60E-07	--	--	--	--	--	--	--	--
10:53414779	rs185736000	<i>PCDH15</i>	G/A	-0.48	0.09	1.10E-07	--	--	--	--	--	--	--	--
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.55	0.10	3.10E-08	--	--	--	--	--	--	--	--
11:5078417	rs77362408	<i>OR52E2</i>	C/G	-0.15	0.03	3.10E-07	0.005	-0.39	0.17	0.02	--	--	--	--
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-0.20	0.04	1.20E-07	--	--	--	--	--	--	--	--
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-0.19	0.04	1.80E-07	--	--	--	--	--	--	--	--
17:32533283	rs140882821	<i>MYO1D</i>	G/A	-0.19	0.04	2.80E-07	0.005	-0.08	0.17	0.61	--	--	--	--
17:78138386	rs144120533	<i>TMC8</i>	C/T	-0.26	0.05	2.70E-07	--	--	--	--	--	--	--	--
18:2770634	--	<i>SMCHD1</i>	G/GT	0.17	0.03	1.40E-07	0.01	0.15	0.13	0.26	--	--	--	--
18:34585879	rs187431535	<i>DTNA</i>	T/A	0.25	0.05	9.20E-08	--	--	--	--	--	--	--	--
18:34712254	rs183229631	<i>DTNA</i>	G/A	0.25	0.05	3.40E-07	--	--	--	--	--	--	--	--
21:37519947	rs11701836	<i>DYRK1A</i>	A/G	-0.03	0.01	4.40E-07	0.28	-0.02	0.02	0.44	0.37	-0.06	0.03	0.02
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	0.03	0.01	1.10E-07	0.45	0.05	0.02	0.02	0.44	-0.01	0.02	0.57
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	-0.31	0.06	2.30E-07	--	--	--	--	--	--	--	--
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	-0.23	0.04	1.40E-07	--	--	--	--	--	--	--	--
X:154877653	rs189305788	<i>F8</i>	C/A	-0.40	0.08	2.40E-07	--	--	--	--	--	--	--	--

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38

^bAAF: Alternate Allele Frequency

Table S6: Results of the mediation analyses for the most associated single nucleotide variants in the regions detected at the genome-wide level ($P < 2 \times 10^{-8}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts

MarkerID ^a	rsID	Closest Gene	Ref/Alt Allele	HbA1c (N=10,338)			FG ^b (N=10,185)			HB ^c (N=6,411)			Class
				Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	
7:44186502	rs2971670	<i>GCK</i>	C/T	0.04	0.01	1.70E-09	0.03	0.01	2.70E-05	0.04	0.01	4.60E-08	Glycemic
10:69334748	rs17476364	<i>HK1</i>	T/C	-0.09	0.01	3.10E-21	-0.09	0.01	1.60E-22	-0.09	0.01	3.40E-17	Unclassified
11:5464062	rs1039215	<i>HBG2/HBE1</i>	A/G	-0.21	0.04	2.00E-09	-0.23	0.13	2.60E-11	-0.21	0.04	7.50E-08	Unclassified
17:82739582	rs113373052	<i>FN3K</i>	C/T	0.03	0.01	4.50E-10	0.03	0.01	1.30E-11	0.03	0.01	2.20E-07	Unclassified
X:154533025	rs76723693	<i>G6PD</i>	A/G	-0.44	0.07	2.16E-09	-0.43	0.07	7.13E-10	-0.40	0.09	7.64E-06	Unclassified
X:154536002	rs1050828	<i>G6PD</i>	C/T	-0.41	0.01	5.10E-210	-0.40	0.01	7.00E-229	-0.42	0.02	2.40E-156	Unclassified

Table S6 (continued):

MarkerID ^a	rsID	Closest Gene	Ref/Alt Allele	MCHC ^d (N=3,779)			MCH ^e (N=3,779)			MCV ^f (N=6,178)			Class
				Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	
7:44186502	rs2971670	<i>GCK</i>	C/T	0.05	0.01	2.20E-06	0.05	0.01	2.60E-06	0.04	0.01	3.90E-08	Glycemic
10:69334748	rs17476364	<i>HK1</i>	T/C	-0.08	0.02	1.30E-07	-0.08	0.02	3.20E-07	-0.09	0.01	2.00E-17	Unclassified
11:5464062	rs1039215	<i>HBG2/HBE1</i>	A/G	-0.21	0.04	1.00E-06	-0.24	0.04	1.50E-08	-0.25	0.04	1.30E-09	Unclassified
17:82739582	rs113373052	<i>FN3K</i>	C/T	0.03	0.01	1.80E-04	0.03	0.01	1.30E-04	0.03	0.01	1.50E-07	Unclassified
X:154533025	rs76723693	<i>G6PD</i>	A/G	-0.44	0.09	6.54E-07	-0.43	0.09	9.32E-07	-0.40	0.09	2.96E-06	Unclassified
X:154536002	rs1050828	<i>G6PD</i>	C/T	-0.41	0.02	7.80E-133	-0.39	0.02	2.40E-124	-0.38	0.02	5.20E-126	Unclassified

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bFG: fasting glucose

^cHB: Hemoglobin

^dMCHC: Mean Corpuscular Hemoglobin Concentration levels

^eMCH: Mean Corpuscular Hemoglobin

^fMCV: Mean Corpuscular Volume

Table S7: Results of the mediation analyses for the most associated single nucleotide variants in the regions detected at the sub-genome-wide level ($P < 5 \times 10^{-7}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts

MarkerID ^a	rsID	Closest Gene	Ref/Alt	HbA1c (N=10,338)			FG ^b (N=10,185)			HB ^c (N=6,411)			Class
				Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	
1:69561493	rs546782425	<i>LRRC7</i>	G/A	-0.37	0.07	4.10E-07	-0.32	0.07	5.62E-06	-0.39	0.08	1.31E-06	Unclassified
2:168900844	rs1402837	<i>G6PC2</i>	C/T	0.03	0.01	1.40E-07	0.02	0.01	3.57E-04	0.03	0.01	3.33E-06	Glycemic
3:5940489	rs541283713	<i>EDEM1</i>	G/T	-0.48	0.09	8.90E-08	-0.49	0.09	1.20E-08	-0.52	0.10	1.50E-07	Unclassified
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	-0.03	0.01	8.90E-08	-0.03	0.01	2.10E-06	-0.03	0.01	2.38E-05	Unclassified
4:100766435	rs184930483	<i>EMCN</i>	C/T	-0.33	0.06	6.50E-08	0.27	0.06	2.33E-06	--	--	--	Unclassified
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-0.32	0.06	4.70E-07	-0.29	0.06	3.92E-06	-0.37	0.08	2.24E-06	Unclassified
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	-0.35	0.07	2.40E-07	-0.30	0.07	8.40E-06	-0.41	0.08	2.99E-07	Unclassified
7:117730990	--	<i>CTTNBP2</i>	TG/T	-0.35	0.07	2.40E-07	-0.30	0.07	8.40E-06	-0.41	0.08	2.99E-07	Unclassified
7:117734487	rs115069847	<i>CTTNBP2</i>	G/A	0.34	0.07	3.90E-07	0.29	0.07	9.37E-06	0.39	0.08	5.89E-07	Unclassified
8:57797437	rs182571425	<i>FAM110B</i>	G/T	0.35	0.06	6.60E-08	0.31	0.06	4.98E-07	0.27	0.08	7.24E-04	Unclassified
8:59954108	rs148165085	<i>CA8</i>	G/C	0.40	0.08	1.60E-07	0.37	0.07	4.81E-07	0.44	0.09	5.91E-07	Unclassified
10:53414779	rs185736000	<i>PCDH15</i>	G/A	-0.48	0.09	1.10E-07	-0.45	0.09	3.87E-07	-0.48	0.11	8.86E-06	Unclassified
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.55	0.10	3.10E-08	-0.53	0.10	3.70E-08	-0.58	0.11	1.16E-07	Unclassified
11:5078417	rs77362408	<i>OR52E2</i>	G/C	0.15	0.03	3.10E-07	0.16	0.03	4.11E-09	0.14	0.03	3.29E-05	Unclassified
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-0.20	0.04	1.20E-07	-0.21	0.04	4.01E-09	-0.20	0.04	2.99E-06	Unclassified
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-0.19	0.04	1.80E-07	-0.20	0.04	2.04E-08	-0.17	0.04	4.53E-05	Unclassified
17:32533283	rs140882821	<i>MYO1D</i>	G/A	-0.19	0.04	2.80E-07	-0.18	0.04	4.48E-07	-0.22	0.04	9.28E-08	Unclassified
17:78138386	rs144120533	<i>TMC8</i>	C/T	-0.26	0.05	2.70E-07	-0.23	0.05	3.18E-06	-0.28	0.06	5.44E-07	Unclassified
18:2770634	--	<i>SMCHD1</i>	GT/G	-0.17	0.03	1.40E-07	-0.15	0.03	7.18E-07	-0.14	0.04	3.40E-04	Unclassified
18:34585879	rs187431535	<i>DTNA</i>	T/A	0.25	0.05	9.20E-08	0.23	0.05	3.03E-07	0.28	0.06	2.06E-06	Unclassified
18:34712254	rs183229631	<i>DTNA</i>	G/A	0.25	0.05	3.40E-07	0.24	0.05	3.99E-07	0.27	0.06	7.71E-06	Unclassified
21:37519947	rs11701836	<i>DYRK1A</i>	G/A	0.03	0.01	4.40E-07	0.03	0.01	6.34E-09	0.02	0.01	1.11E-03	Unclassified
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	0.03	0.01	1.10E-07	0.03	0.00	1.02E-08	0.02	0.01	2.98E-04	Unclassified
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	-0.31	0.06	2.30E-07	-0.32	0.06	2.33E-08	-0.33	0.07	2.93E-06	Unclassified
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	-0.23	0.04	1.40E-07	-0.24	0.04	2.59E-08	-0.23	0.05	1.24E-05	Unclassified
X:154877653	rs189305788	<i>F8</i>	C/A	-0.40	0.08	2.40E-07	-0.40	0.07	9.30E-08	-0.38	0.09	1.36E-05	Unclassified

Table S7 (continued):

MarkerID ^a	rsID	Closest Gene	Ref/Alt	MCHC ^d (N=3,779)			MCH ^e (N=3,779)			MCV ^f (N=6,178)			Class
				Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	
1:69561493	rs546782425	<i>LRRC7</i>	G/A	-0.40	0.08	1.32E-06	-0.38	0.08	4.55E-06	-0.36	0.08	3.34E-06	Unclassified
2:168900844	rs1402837	<i>G6PC2</i>	C/T	0.03	0.01	1.00E-03	0.03	0.01	1.06E-03	0.03	0.01	2.22E-06	Glycemic
3:5940489	rs541283713	<i>EDEM1</i>	G/T	-0.46	0.10	1.08E-05	-0.45	0.10	1.21E-05	-0.43	0.10	1.16E-05	Unclassified
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	-0.02	0.01	8.33E-02	-0.02	0.01	1.14E-01	-0.03	0.01	7.46E-05	Unclassified
4:100766435	rs184930483	<i>EMCN</i>	C/T	--	--	--	--	--	--	--	--	--	Unclassified
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-0.36	0.08	5.33E-06	-0.35	0.08	7.73E-06	-0.33	0.08	1.33E-05	Unclassified
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	-0.35	0.08	4.25E-05	-0.32	0.08	1.01E-04	-0.32	0.08	3.48E-05	Unclassified
7:117730990	--	<i>CTTNBP2</i>	TG/T	-0.35	0.08	4.25E-05	-0.32	0.08	1.01E-04	-0.32	0.08	3.48E-05	Unclassified
7:117734487	rs115069847	<i>CTTNBP2</i>	G/A	0.33	0.08	7.19E-05	0.31	0.08	1.72E-04	0.31	0.08	6.14E-05	Unclassified
8:57797437	rs182571425	<i>FAM110B</i>	G/T	--	--	--	--	--	--	0.27	0.08	5.29E-04	Unclassified
8:59954108	rs148165085	<i>CA8</i>	G/C	0.44	0.10	8.96E-06	0.41	0.10	1.91E-05	0.39	0.09	1.74E-05	Unclassified
10:53414779	rs185736000	<i>PCDH15</i>	G/A	-0.30	0.13	1.87E-02	-0.28	0.12	2.16E-02	-0.33	0.12	5.39E-03	Unclassified
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.48	0.12	4.50E-05	-0.47	0.12	4.80E-08	-0.46	0.11	6.80E-05	Unclassified
11:5078417	rs77362408	<i>OR52E2</i>	G/C	0.13	0.03	1.07E-04	0.16	0.03	1.75E-06	0.17	0.03	5.64E-07	Unclassified
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-0.21	0.04	3.52E-06	-0.24	0.04	9.30E-08	-0.23	0.04	4.11E-08	Unclassified
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-0.18	0.04	3.64E-05	-0.20	0.04	5.91E-06	-0.18	0.04	2.18E-05	Unclassified
17:32533283	rs140882821	<i>MYO1D</i>	G/A	-0.22	0.04	5.47E-07	-0.21	0.04	6.67E-07	-0.21	0.04	7.39E-07	Unclassified
17:78138386	rs144120533	<i>TMC8</i>	C/T	-0.25	0.08	2.51E-03	-0.23	0.08	4.83E-03	-0.27	0.06	1.53E-06	Unclassified
18:2770634	--	<i>SMCHD1</i>	GT/G	-0.14	0.04	8.07E-04	-0.15	0.04	1.89E-04	-0.15	0.04	1.66E-04	Unclassified
18:34585879	rs187431535	<i>DTNA</i>	T/A	0.33	0.08	1.45E-05	0.33	0.08	1.82E-05	0.29	0.06	1.37E-06	Unclassified
18:34712254	rs183229631	<i>DTNA</i>	G/A	0.32	0.08	6.10E-05	0.31	0.08	8.36E-05	0.28	0.06	4.57E-06	Unclassified
21:37519947	rs11701836	<i>DYRK1A</i>	G/A	0.03	0.01	1.20E-03	0.03	0.01	1.51E-03	0.02	0.01	2.74E-03	Unclassified
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	0.02	0.01	4.02E-03	0.02	0.01	2.96E-02	0.02	0.01	9.61E-03	Unclassified
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	-0.33	0.07	2.08E-06	-0.31	0.07	8.07E-06	-0.30	0.07	9.56E-06	Unclassified
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	-0.25	0.05	4.68E-06	-0.24	0.05	4.00E-06	-0.23	0.05	7.27E-06	Unclassified
X:154877653	rs189305788	<i>F8</i>	C/A	-0.42	0.09	1.49E-06	-0.40	0.09	2.29E-06	-0.37	0.08	6.53E-06	Unclassified

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bFG: fasting glucose

^cHB: Hemoglobin

^dMCHC: mean corpuscular hemoglobin concentration levels

^eMCH: mean corpuscular hemoglobin

^fMCV: mean corpuscular volume

Table S8: Association of the most associated single nucleotide variants in the regions detected at the genome-wide level ($P < 2 \times 10^{-8}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts with fasting glucose (FG, N=26,883) and erythrocytic traits (HCT, HB, MCV, MCH, MCHC, RBC, RDW, N=25,080)

				HbA1c		FG ^c		HB ^d		HCT ^e		MCV ^f	
MarkerID ^a	rsID	Closest Gene	Ref/Alt Allele	Beta	P value	Beta	P value	Score ^b	P value	Score ^b	P value	Score ^b	P value
7:44186502	rs2971670	<i>GCK</i>	C/T	0.04	1.70E-09	0.06	7.40E-28	33.37	0.68	7.79	0.78	-2.35	0.87
10:69334748	rs17476364	<i>HK1</i>	T/C	-0.09	3.10E-21	-0.01	0.17	420.08	1.60E-12	140.77	2.10E-12	35.6	4.60E-04
11:5464062	rs1039215	<i>HBG2</i>	A/G	-0.21	2.00E-09	-0.02	0.41	-49.73	4.90E-04	-20.35	3.60E-05	-5.75	0.02
17:82739582	rs113373052	<i>FN3K</i>	C/T	0.03	4.50E-10	0.003	0.5	59.46	0.54	39.33	0.23	-11.41	0.48
X:154533025	rs76723693	<i>G6PD</i>	A/G	-0.44	0.07	-0.09	0.10	-12.50	0.05	-2.69	0.22	1.85	0.08
X:154536002	rs1050828	<i>G6PD</i>	C/T	-0.41	5.10E-210	-0.01	0.42	-246.54	3.40E-13	-76.5	6.30E-11	66.83	2.90E-31

Table S8 (continued):

				MCH ^g		MCHC ^h		RBC ⁱ		RDW ^j		
MarkerID*	rsID	Closest Gene	Ref/Alt Allele	Score ^b	P value	Score ^b	P value	Score ^b	P value	Score ^b	P value	Class
7:44186502	rs2971670	<i>GCK</i>	C/T	-21.06	0.54	62.55	0.37	91.29	0.57	91.04	0.03	Glycemic
10:69334748	rs17476364	<i>HK1</i>	T/C	64.16	0.01	-28.79	0.55	267.44	0.02	35.47	0.23	Erythrocytic
11:5464062	rs1039215	<i>HBG2</i>	A/G	-15.07	0.01	7.2	0.65	-11.97	0.71	16.87	0.06	Unclassified
17:82739582	rs113373052	<i>FN3K</i>	C/T	-55.38	0.16	-142.06	0.08	331.35	0.08	-54.06	0.28	Unclassified
X:154533025	rs76723693	<i>G6PD</i>	A/G	2.30	0.39	-12.20	0.09	-41.38	0.004	-15.84	1.46E-04	Erythrocytic
X:154536002	rs1050828	<i>G6PD</i>	C/T	122.46	1.10E-16	-152.61	6.20E-05	-911.53	1.90E-32	-248.94	6.30E-34	Erythrocytic

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bfrom a pooled ancestry score test

^cFG: Fasting Glucose

^dHB: Hemoglobin

^eHCT: Hematocrit,

^fMCV: Mean Corpuscular Volume,

^gMCH: Mean Corpuscular Hemoglobin

^hMCHC: Mean Corpuscular Hemoglobin Concentration

ⁱRBC: Red Blood Cell count

^jRDW: Red cell Distribution Width

Table S9: Association of the most associated single nucleotide variants in the regions detected at the genome-wide level ($P < 2 \times 10^{-8}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts with erythrocytic traits (HCT, HB, MCV, MCH, RBC, RDW) in published genome-wide association studies composed of people of European, Hispanic and African ancestry

Ancestry	PMID	Trait	Effect/Other Allele	EAF ^a	Beta	SE	<i>P</i> value
rs17476364 (10:69,334,748) <i>HK1</i>							
European	27863252	HCT ^b	C/T	0.11	0.15	0.006	7.7E-159
European	27863252	HB ^c	C/T	0.11	0.15	0.006	1.2E-156
European	27863252	MCH ^d	C/T	0.11	0.08	0.006	3.7E-44
European	27863252	MCV ^e	C/T	0.11	0.09	0.006	7.3E-55
European	27863252	RBC ^f	C/T	0.11	0.08	0.006	1.8E-48
European	27863252	RDW ^g	C/T	0.11	0.04	0.006	2.0E-11
rs1050828 (X:154,536,002) <i>G6PD</i>							
African American	21153663	HCT	T/C	0.11	-0.22	0.03	1.2E-13
African American	21153663	HB	T/C	0.11	-0.24	0.03	1.2E-15
Hispanic	28453575	MCV	T/C	0.02	1.92	0.22	1.3E-17
African American	21153663	MCV	T/C	0.11	0.34	0.04	3.0E-18
African American	23446634	RBC	T/C	0.11	-0.42	0.04	4.3E-21
Hispanic	28453575	RBC	T/C	0.02	-0.13	0.01	1.8E-18
Hispanic	28453575	RDW	T/C	0.02	-0.04	0.003	1.5E-29

^aEAF: Effect Allele Frequency

^bHCT: Hematocrit

^cHB: hemoglobin

^dMCH: mean corpuscular hemoglobin

^eMCV: Mean Corpuscular Volume

^fRBC: Red Blood Cell count

^gRDW: Red cell Distribution Width

Table S10: Association of the most associated single nucleotide variants in the regions detected at the sub-genome-wide level ($P < 5 \times 10^{-7}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts with fasting glucose (FG) in the TOPMed Diabetes working group (N=26,883) and erythrocytic traits (HCT, HB, MCV, MCH, MCHC, RBC, RDW) in the TOPMed Hematology working group (N=25,080)

MarkerID ^a	rsID	Closest Gene	Ref/Alt	HbA1c		FG ^c		HB ^d		HCT ^e		MCV ^f	
				Beta	P value	Beta	P value	Score ^b	P value	Score ^b	P value	Score ^b	P value
1:69561493	rs546782425	<i>LRRC7</i>	G/A	-0.37	4.10E-07	-0.03	5.10E-01	-13.65	3.90E-02	-4.37	5.60E-02	1.74	1.30E-01
2:168900844	rs1402837	<i>G6PC2</i>	C/T	0.03	1.40E-07	0.04	3.40E-17	-2.77	9.80E-01	-3.85	9.00E-01	-11.70	4.40E-01
3:5940489	rs541283713	<i>EDEM1</i>	G/T	-0.48	8.90E-08	-0.04	4.90E-01	-3.06	6.40E-01	-2.05	3.60E-01	-0.09	9.30E-01
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	-0.03	8.90E-08	-0.002	7.60E-01	81.14	3.40E-01	17.81	5.30E-01	14.51	3.10E-01
4:100766435	rs184930483	<i>EMCN</i>	T/C	-0.33	6.50E-08	-0.13	7.20E-02	--	--	--	--	--	--
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-0.32	4.70E-07	-0.01	7.60E-01	-17.70	4.50E-02	-5.77	5.90E-02	-0.91	5.50E-01
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	-0.35	2.40E-07	0.02	6.80E-01	1.52	8.30E-01	0.59	8.10E-01	2.76	1.50E-02
7:117730990	--	<i>CTTNBP2</i>	TG/T	-0.35	2.40E-07	0.02	6.80E-01	1.52	8.30E-01	0.59	8.10E-01	2.76	1.50E-02
7:117734487	rs115069847	<i>CTTNBP2</i>	A/G	-0.34	3.90E-07	0.03	6.00E-01	0.71	9.20E-01	0.33	8.90E-01	2.82	1.40E-02
8:57797437	rs182571425	<i>FAM110B</i>	T/G	-0.35	6.60E-08	-0.02	6.90E-01	8.95	3.00E-01	3.34	2.50E-01	1.18	3.50E-01
8:59954108	rs148165085	<i>CA8</i>	C/G	-0.40	1.60E-07	-0.02	6.40E-01	-3.57	6.30E-01	-1.35	6.00E-01	0.43	7.10E-01
10:53414779	rs185736000	<i>PCDH15</i>	G/A	-0.48	1.10E-07	-0.007	9.30E-01	-18.85	1.00E-03	-6.37	1.30E-03	0.22	8.00E-01
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.55	3.10E-08	-0.01	0.85	2.98	0.56	1.63	0.36	-0.26	0.76
11:5078417	rs77362408	<i>OR52E2</i>	C/G	-0.15	3.10E-07	0.03	2.80E-01	-56.71	9.60E-04	-22.80	1.20E-04	-11.35	7.90E-05
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-0.20	1.20E-07	0.02	5.00E-01	-45.16	8.40E-04	-18.46	7.80E-05	-5.04	2.40E-02
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-0.19	1.80E-07	0.01	6.30E-01	-21.24	1.30E-01	-10.67	2.70E-02	-1.81	4.20E-01
17:32533283	rs140882821	<i>MYO1D</i>	G/A	-0.19	2.80E-07	-0.04	2.00E-01	-12.32	3.30E-01	-3.20	4.70E-01	2.30	2.90E-01
17:78138386	rs144120533	<i>TMC8</i>	C/T	-0.26	2.70E-07	-0.08	1.30E-01	0.30	9.70E-01	0.55	8.60E-01	4.02	1.10E-02
18:2770634	--	<i>SMCHD1</i>	G/GT	0.17	1.40E-07	0.04	8.00E-02	30.21	5.90E-02	10.26	6.20E-02	0.65	8.10E-01
18:34585879	rs187431535	<i>DTNA</i>	T/A	0.25	9.20E-08	0.02	6.60E-01	-16.25	1.70E-01	-3.10	4.40E-01	-1.30	4.60E-01
18:34712254	rs183229631	<i>DTNA</i>	G/A	0.25	3.40E-07	0.01	8.20E-01	-13.07	2.70E-01	-1.92	6.30E-01	-1.23	4.80E-01
21:37519947	rs11701836	<i>DYRK1A</i>	A/G	-0.03	4.40E-07	-0.004	3.80E-01	125.48	1.80E-01	26.06	4.10E-01	-9.34	5.40E-01
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	0.03	1.10E-07	0.005	2.90E-01	-1006.23	1.50E-23	-243.33	7.70E-13	-172.78	2.30E-24
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	-0.31	2.30E-07	-0.06	1.70E-01	-2.48	7.60E-01	-1.40	6.20E-01	3.42	1.70E-02
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	-0.23	1.40E-07	-0.04	2.50E-01	-10.06	3.10E-01	-3.41	3.20E-01	1.82	2.90E-01
X:154877653	rs189305788	<i>F8</i>	C/A	-0.40	2.40E-07	-0.09	9.10E-02	-10.14	1.30E-01	-2.25	3.30E-01	2.10	7.00E-02

Table S10 (continued):

MarkerID ^a	rsID	Closest Gene	Ref/Alt Allele	MCH ^s		MCHC ^h		RBC ⁱ		RDW ^j		Class
				Score ^b	<i>P</i> value	Score ^b	<i>P</i> value	Score ^b	<i>P</i> value	Score ^b	<i>P</i> value	
1:69561493	rs546782425	<i>LRRC7</i>	G/A	4.88	8.60E-02	-3.55	6.40E-01	-42.23	4.50E-03	-7.04	1.10E-01	Erythrocytic
2:168900844	rs1402837	<i>G6PC2</i>	C/T	-28.09	4.60E-01	-50.20	5.20E-01	295.90	9.40E-02	18.65	6.90E-01	Glycemic
3:5940489	rs541283713	<i>EDEM1</i>	G/T	1.10	6.90E-01	11.35	9.00E-02	-16.87	2.30E-01	-2.05	6.40E-01	Unclassified
3:73834413	rs73103979	<i>PDZRN3</i>	C/T	38.39	2.70E-01	71.65	2.90E-01	-135.03	4.00E-01	-41.56	3.30E-01	Unclassified
4:100766435	rs184930483	<i>EMCN</i>	T/C	--	--	--	--	--	--	--	--	Unclassified
5:73310829	rs148873180	<i>FOXD1</i>	G/A	-2.42	5.40E-01	-4.85	6.30E-01	-26.00	2.00E-01	-0.71	8.90E-01	Unclassified
7:117730753	rs77974976	<i>CTTNBP2</i>	G/T	5.60	4.40E-02	4.20	5.60E-01	-40.12	5.90E-03	-0.58	8.90E-01	Unclassified
7:117730990	--	<i>CTTNBP2</i>	TG/T	5.60	4.40E-02	4.20	5.60E-01	-40.12	5.90E-03	-0.58	8.90E-01	Unclassified
7:117734487	rs115069847	<i>CTTNBP2</i>	A/G	5.67	4.40E-02	3.65	6.20E-01	-41.33	5.00E-03	-0.79	8.60E-01	Unclassified
8:57797437	rs182571425	<i>FAM110B</i>	T/G	1.25	6.60E-01	-0.98	8.70E-01	-8.07	5.40E-01	-1.82	6.50E-01	Unclassified
8:59954108	rs148165085	<i>CA8</i>	C/G	0.18	9.50E-01	-5.88	4.30E-01	2.58	8.70E-01	1.13	8.00E-01	Unclassified
10:53414779	rs185736000	<i>PCDH15</i>	G/A	0.32	8.90E-01	0.39	9.50E-01	-21.08	8.40E-02	1.75	5.80E-01	Erythrocytic
10:108997093	rs551601853	<i>XPNPEP1</i>	A/G	-0.35	0.87	-4.87	0.4	8.82	0.44	-4.14	0.18	Unclassified
11:5078417	rs77362408	<i>OR52E2</i>	C/G	-24.54	8.90E-04	8.94	6.40E-01	39.43	3.10E-01	29.01	8.60E-03	Erythrocytic
11:5492424	rs73392143	<i>HBG2/HBE1</i>	G/T	-12.84	2.60E-02	8.86	5.60E-01	-14.88	6.20E-01	12.08	1.60E-01	Erythrocytic
11:6014322	rs76128436	<i>OR56A4</i>	C/T	-3.24	5.80E-01	15.99	3.00E-01	-28.05	3.60E-01	9.26	2.80E-01	Unclassified
17:32533283	rs140882821	<i>MYO1D</i>	G/A	3.39	5.40E-01	-0.40	9.80E-01	-13.13	6.50E-01	-14.39	9.30E-02	Unclassified
17:78138386	rs144120533	<i>TMC8</i>	C/T	9.74	1.10E-02	4.00	5.80E-01	-21.21	2.10E-01	-8.72	9.80E-02	Unclassified
18:2770634	--	<i>SMCHD1</i>	G/GT	2.95	6.70E-01	9.26	5.90E-01	33.09	3.50E-01	2.04	8.30E-01	Unclassified
18:34585879	rs187431535	<i>DTNA</i>	T/A	-4.56	2.90E-01	-6.49	4.50E-01	-9.72	6.20E-01	1.23	8.30E-01	Unclassified
18:34712254	rs183229631	<i>DTNA</i>	G/A	-3.79	3.70E-01	-5.50	5.10E-01	-11.17	5.60E-01	-1.79	7.60E-01	Unclassified
21:37519947	rs11701836	<i>DYRK1A</i>	A/G	-23.17	5.40E-01	85.42	2.80E-01	185.59	3.00E-01	-80.01	9.40E-02	Unclassified
22:37066896	rs855791	<i>TMPRSS6</i>	G/A	-493.31	1.80E-32	-611.28	8.40E-14	218.78	2.60E-01	361.15	1.50E-12	Erythrocytic
X:153542357	rs143745197	<i>ATP2B3</i>	G/A	9.92	5.50E-03	4.22	6.50E-01	-42.99	2.20E-02	-14.61	3.80E-03	Erythrocytic
X:154593327	rs184539426	<i>CTAG1A</i>	G/T	3.53	4.20E-01	-4.71	6.80E-01	-43.98	5.70E-02	-15.30	2.10E-02	Unclassified
X:154877653	rs189305788	<i>F8</i>	C/A	3.25	2.70E-01	-8.98	2.40E-01	-43.17	5.70E-03	-16.90	1.60E-04	Erythrocytic

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bfrom a pooled ancestry score test

^sMCH: mean corpuscular hemoglobin

^hMCHC: mean corpuscular hemoglobin concentration

ⁱRBC: red blood cell count

^jRDW: red cell distribution width

Table S11: Gene-based and burden tests performed in the regions detected at the genome-wide level ($P < 2 \times 10^{-8}$) in the meta-analysis of HbA1c in non-diabetic individuals in TOPMed cohorts and classified as erythrocytic

SKAT								
	Ancestry	Chr	Ensembl gene id	Number of variants ^a	n.sample.alt ^b	Q	P value	Annotation
<i>HK1</i>	Europeans	10	ENSG00000156515	37	48	255403.79	0.59	missense
				2	2	11847.45	0.33	high confidence loss of function
				30	173	659814.94	0.68	synonymous
<i>HBG2</i>	African Americans	11	ENSG00000196565	5	54	37503.20	0.73	missense
				2	3	1572.69	0.84	high confidence loss of function
				3	7	9253.68	0.69	synonymous
<i>G6PD</i>	African Americans	X	ENSG00000160211	15	76	4320297.73	9.72E-11	missense
				--	--	--	--	high confidence loss of function
				11	34	92231.24	0.82	synonymous

Burden										
	Ancestry	Chr	Ensembl gene id	Number of variants ^a	n.sample.alt ^b	burden.skew ^c	Beta	SE	P value	Annotation
<i>HK1</i>	Europeans	10	ENSG00000156515	37	48	11.19	0.001	0.002	0.70	missense
				2	2	55.45	-0.015	0.010	0.13	high confidence loss of function
				30	173	6.66	0.001	0.001	0.42	synonymous
<i>HBG2</i>	African Americans	11	ENSG00000196565	5	54	7.45	-0.002	0.003	0.52	missense
				2	3	32.20	0.004	0.010	0.69	high confidence loss of function
				3	7	21.04	-0.004	0.007	0.57	synonymous
<i>G6PD</i>	African Americans	X	ENSG00000160211	15	76	7.41	-0.007	0.002	4.69E-05	missense
				--	--	--	--	--	--	high confidence loss of function
				11	34	11.23	0.003	0.002	0.19	synonymous

^avariants with MAF < 1% were included in the test

^bThe number of samples with an observed alternate allele at any variant in the aggregate set

^cThe skewness of the burden value for all samples

Table S12: Analysis of HbA1c associations of the top single nucleotide variants in *HGB2* and *G6PD* before and after conditioning on the sickle cell trait variant (rs334) in non-diabetic African-Americans in TOPMed cohorts

MarkerID ^a	Closest Gene	Ref/Alt Alleles	EAf ^b	Beta	SE	<i>P</i> value
Main analysis						
11:5464062 (rs1039215)	<i>HBG2/HBE1</i>	A/G	0.03	-0.21	0.04	2.04E-09
X:154533025 (rs76723693)	<i>G6PD</i>	A/G	0.01	-0.44	0.07	2.16E-09
X:154536002 (rs1050828)	<i>G6PD</i>	C/T	0.12	-0.41	0.01	8.38E-205
Adjusted on rs334						
11:5464062 (rs1039215)	<i>HBG2/HBE1</i>	A/G	0.03	-0.14	0.04	1.22E-03
X:154533025 (rs76723693)	<i>G6PD</i>	A/G	0.01	-0.45	0.07	8.29E-10
X:154536002 (rs1050828)	<i>G6PD</i>	C/T	0.12	-0.41	0.01	5.75E-210

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bEAf: Effect Allele Frequency

Table S13: Analysis of HbA1c associations of haplotypes constructed with the distinct single nucleotide variants in the *HBB-HBG2* locus

	rs334	rs1039215	haplotype frequency	Beta	SE	<i>P</i> value
reference haplotype	T	A	0.95	--	--	--
haplo.1	A	A	0.02	-0.12	0.04	5.24E-03
haplo.2	A	G	0.02	-0.21	0.04	5.54E-08
haplo.3	T	G	0.006	-0.18	0.08	1.82E-02

Table S14: Distribution of HbA1c in the 3,123 African-Americans from TOPMed according to genotypes at the two *G6PD* variants (rs1050828 & rs76723693) and at the *HBG2* variant (rs1039215)

Genotype	Variable	Obs	Mean	SD	Min	Max
rs1050828=0 & rs1039215=0	hba1c	2,372	5.65	0.39	3.7	6.4
rs1050828=0 & rs1039215=1	hba1c	138	5.42	0.44	3.8	6.4
rs1050828=1 & rs1039215=0	hba1c	375	5.28	0.46	3.6	6.4
rs1050828=1 & rs1039215=1	hba1c	15	5.27	0.61	4.5	6.2
rs1050828=2 & rs1039215=0	hba1c	152	4.78	0.40	3.6	6.3
rs1050828=2 & rs1039215=1	hba1c	12	4.60	0.41	4.0	5.5
rs76723693=0 & rs1039215=0	hba1c	2,897	5.56	0.46	3.6	6.4
rs76723693=0 & rs1039215=1	hba1c	167	5.35	0.50	3.8	6.4
rs76723693=1 & rs1039215=0	hba1c	19	5.09	0.39	4.3	6.0
rs76723693=1 & rs1039215=1	hba1c	1	4.90	--	4.9	4.9
rs76723693=2 & rs1039215=0	hba1c	5	4.62	0.13	4.5	4.8

Table S15: Estimated number of African Americans adults with type 2 diabetes in the United States whose diagnosis would be missed due to the common and rare glucose-6-phosphate dehydrogenase (*G6PD*) variants if screened with HbA1c

AFRICAN AMERICAN					
rs1050828					
Sex	Adjusted HbA1c thresholds ^a	Estimated proportion with HbA1c above the adjusted threshold* but below the standard clinical threshold of 6.5 %-units (%) for diabetes diagnosis	Genotype	Expected proportion with genotype (%)	Estimated proportion with missed diabetes diagnosis (%)
Men	5.62-6.49 %-units	13.59 [11.42-15.75]	T	12.00	1.63
Women	5.62-6.49 %-units	14.19 [11.38-17.00]	TT	1.40	0.20
Women	6.16-6.49 %-units	2.31 [0.13-3.32]	CT	21.10	0.49
Total (%)					2.32
Total (million)^b					0.70
rs76723693					
Sex	Adjusted HbA1c thresholds	Estimated proportion with HbA1c above the adjusted threshold* but below the standard clinical threshold of 6.5 %-units (%) for diabetes diagnosis	Genotype	Expected proportion with genotype (%)	Estimated proportion with missed diabetes diagnosis (%)
Men	5.52-6.49 %-units	17.25 [15.03-19.47]	G	0.50	0.09
Women	5.52-6.49 %-units	18.32 [14.86-21.78]	GG	2.5E-04	4.6x10 ⁻⁴
Women	6.04-6.49 %-units	3.58 [2.33-4.82]	AG	1.00	0.04
Total (%)					0.13
Total (million)^b					0.04
rs1050828 & rs76723693					
Total (%)					2.45
Total (million)^b					0.74

^aAdjusted HbA1c thresholds to define diabetes were calculated by subtracting the sex and genotype specific effect of each variant from the standard clinical threshold of 6.5 %-units.

^bThe estimated number of African American adults in the United States is 30.14 million (2016 United States Census Bureau).

We restricted the NHANES analytic sample to 1,227 adults aged ≥ 18 years, who self-identified as Non-Hispanic Black.

In African Americans, the mean age was 44.2 (42.9, 45.6) years, 55.4% (52.5, 58.3) were women, and mean HbA1c, excluding those with physician-diagnosed diabetes by self-report or medication use, was 5.56 (5.52, 5.59) %-units. Estimated proportion with diagnosed diabetes = 14.56 (11.73, 17.9) %. Estimated proportion with undiagnosed diabetes by HbA1c ≥ 6.5 %-units = 2.56 (1.89, 3.24) %.

Table S16: Estimated number of Hispanic adults with type 2 diabetes in the United States whose diagnosis would be missed due to the common and rare glucose-6-phosphate dehydrogenase (*G6PD*) variants if screened with HbA1c

HISPANIC					
rs1050828					
Sex	Adjusted HbA1c thresholds^a	Estimated proportion with HbA1c above the adjusted threshold* but below the standard clinical threshold of 6.5 %-units (%) for diabetes diagnosis	Genotype	Expected proportion with genotype (%)	Estimated proportion with missed diabetes diagnosis (%)
Men	5.62-6.49 %-units	11.14 [9.28-13.01]	T	2.00	0.22
Women	5.62-6.49 %-units	10.56 [8.70-12.42]	TT	4.0E-02	4.0x10 ⁻³
Women	6.16-6.49 %-units	0.11 [0.54-1.68]	CT	3.90	0.04
Total (%)					0.26
Total (million)^b					0.10

^aAdjusted HbA1c thresholds to define diabetes were calculated by subtracting the sex and genotype specific effect of each variant from the standard clinical threshold of 6.5 %-units.

^bThe estimated number of Hispanic adults in the United States is 39.33 million (2016 United States Census Bureau).

We restricted the NHANES analytic sample to 1,768 adults aged ≥ 18 years, who self-identified as Mexican American/Other Hispanic.

In Mexican Americans/Other Hispanics, the mean age was 41.3 (39.4, 43.1) years, 50.4 (48.4, 52.3)% were women, and mean HbA1c was 5.50 (5.45, 5.56) %-units. Estimated proportion with physician-diagnosed diabetes = 13.46 (10.09, 16.83) &. Estimated proportion with undiagnosed diabetes by HbA1c ≥ 6.5 %-units = 2.33 (1.39, 3.28) %.

Table S17: Association results of the TOPMed sub-genome signals in the UK Biobank

MarkerID ^a	rsID	Gene	Alternate Allele	Imputation Info Score	African-ancestry (N=5,311)				European-ancestry (N=398,122)			
					Allele Frequency	Beta	SE	<i>P</i> value	Allele Frequency	Beta	SE	<i>P</i> value
1:69561493	rs546782425	<i>LRRC7</i>	A	0.70	0.01	0.019	0.048	7.00E-01	--	--	--	--
2:168900844	rs1402837	<i>G6PC2</i>	T	1.00	--	--	--	--	0.23	0.023	0.001	4.46E-116
3:5940489	rs541283713	<i>EDEM1</i>	T	0.72	0.004	0.022	0.078	7.83E-01	--	--	--	--
3:73834413	rs73103979	<i>PDZRN3</i>	T	1.00	--	--	--	--	0.25	0.000	0.001	9.25E-01
4:100766435	rs184930483	<i>EMCN</i>	--	--	--	--	--	--	--	--	--	--
5:73310829	rs148873180	<i>FOXD1</i>	A	0.89	0.01	-0.036	0.041	3.74E-01	--	--	--	--
7:117730753	rs77974976	<i>CTTNBP2</i>	T	0.84	0.008	-0.123	0.051	1.57E-02	--	--	--	--
7:117730990	--	<i>CTTNBP2</i>	--	--	--	--	--	--	--	--	--	--
7:117734487	rs115069847	<i>CTTNBP2</i>	G	0.83	0.008	-0.086	0.051	8.94E-02	--	--	--	--
8:57797437	rs182571425	<i>FAM110B</i>	G	0.86	--	--	--	--	0.003	-0.003	0.008	7.05E-01
8:59954108	rs148165085	<i>CA8</i>	G	0.91	0.008	0.038	0.051	4.56E-01	--	--	--	--
10:53414779	rs185736000	<i>PCDH15</i>	C	0.59	--	--	--	--	--	--	--	--
10:108997093	rs551601853	<i>XPNPEP1</i>	G	0.64	5.28E-04	-0.073	0.249	7.69E-01	--	--	--	--
11:5078417	rs77362408	<i>OR52E2</i>	G	0.95	0.02	0.037	0.035	2.84E-01	--	--	--	--
11:5492424	rs73392143	<i>HBG2/HBE1</i>	T	0.98	0.01	-0.017	0.042	6.86E-01	--	--	--	--
11:6014322	rs76128436	<i>OR56A4</i>	T	0.94	0.02	0.004	0.033	9.04E-01	--	--	--	--
17:32533283	rs140882821	<i>MYO1D</i>	A	0.73	0.02	-0.029	0.037	4.42E-01	--	--	--	--
17:78138386	rs144120533	<i>TMC8</i>	T	1.00	--	--	--	--	0.003	-0.145	0.008	1.10E-79
18:2770634	--	<i>SMCHD1</i>	--	--	--	--	--	--	--	--	--	--
18:34585879	rs187431535	<i>DTNA</i>	A	0.92	--	--	--	--	0.005	-0.006	0.006	3.62E-01
18:34712254	rs183229631	<i>DTNA</i>	A	0.94	--	--	--	--	0.005	-0.004	0.006	5.62E-01
21:37519947	rs11701836	<i>DYRK1A</i>	G	0.99	0.33	0.000	0.009	9.65E-01	0.26	0.000	0.001	8.67E-01
22:37066896	rs855791	<i>TMPRSS6</i>	G	0.99	0.87	0.005	0.013	7.19E-01	0.56	-0.024	0.001	7.33E-167
X:153542357	rs143745197	<i>ATP2B3</i>	A	0.94	0.007	-0.210	0.044	2.39E-06	--	--	--	--
X:154593327	rs184539426	<i>CTAG1A</i>	T	0.55	0.007	-0.045	0.060	4.54E-01	--	--	--	--
X:154877653	rs189305788	<i>F8</i>	A	0.64	0.004	-0.183	0.074	1.30E-02	--	--	--	--

Table S17 (continued):

MarkerID ^a	rsID	Gene	Alternate Allele	Imputation Info Score	Asian-ancestry (N=1,331)			
					Allele Frequency	Beta	SE	P value
1:69561493	rs546782425	<i>LRRC7</i>	A	0.70	--	--	--	--
2:168900844	rs1402837	<i>G6PC2</i>	T	1.00	--	--	--	--
3:5940489	rs541283713	<i>EDEM1</i>	T	0.72	--	--	--	--
3:73834413	rs73103979	<i>PDZRN3</i>	T	1.00	--	--	--	--
4:100766435	rs184930483	<i>EMCN</i>	C	0.77	0.02	-0.034	0.047	4.69E-01
5:73310829	rs148873180	<i>FOXD1</i>	A	0.89	--	--	--	--
7:117730753	rs77974976	<i>CTTNBP2</i>	T	0.84	--	--	--	--
7:117730990	--	<i>CTTNBP2</i>	--	--	--	--	--	--
7:117734487	rs115069847	<i>CTTNBP2</i>	G	0.83	--	--	--	--
8:57797437	rs182571425	<i>FAM110B</i>	G	0.86	--	--	--	--
8:59954108	rs148165085	<i>CA8</i>	G	0.91	--	--	--	--
10:53414779	rs185736000	<i>PCDH15</i>	C	0.59	--	--	--	--
10:108997093	rs551601853	<i>XPNPEP1</i>	G	0.64	--	--	--	--
11:5078417	rs77362408	<i>OR52E2</i>	G	0.95	--	--	--	--
11:5492424	rs73392143	<i>HBG2/HBE1</i>	T	0.98	--	--	--	--
11:6014322	rs76128436	<i>OR56A4</i>	T	0.94	--	--	--	--
17:32533283	rs140882821	<i>MYO1D</i>	A	0.73	--	--	--	--
17:78138386	rs144120533	<i>TMC8</i>	T	1.00	--	--	--	--
18:2770634	--	<i>SMCHD1</i>	--	--	--	--	--	--
18:34585879	rs187431535	<i>DTNA</i>	A	0.92	--	--	--	--
18:34712254	rs183229631	<i>DTNA</i>	A	0.94	--	--	--	--
21:37519947	rs11701836	<i>DYRK1A</i>	G	0.99	0.39	-0.029	0.014	4.14E-02
22:37066896	rs855791	<i>TMPRSS6</i>	G	0.99	--	--	--	--
X:153542357	rs143745197	<i>ATP2B3</i>	A	0.94	--	--	--	--
X:154593327	rs184539426	<i>CTAG1A</i>	T	0.55	--	--	--	--
X:154877653	rs189305788	<i>F8</i>	A	0.64	--	--	--	--

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

Table S18: Effect of the *G6PD*-rs76723693 and the *HBG2*-rs1039215 variants in the whole sample of non-diabetic African-American individuals from TOPMed cohorts and in the two largest African-American cohorts (JHS and MESA)

MarkerID ^a	Ref/Alt Allele	N	EAf ^b	AC ^c	Beta	SE	<i>P</i> value	Cohort
11:5464062	A/G	2,356	0.03	139.00	-0.204	0.040	3.74E-07	JHS
11:5464062	A/G	667	0.02	28.00	-0.202	0.078	9.54E-03	MESA
11:5464062	A/G	3,123	0.03	172.00	-0.210	0.035	2.04E-09	JHS+MESA+ARIC
X:154533025	A/G	2,356	0.006	26.28	-0.419	0.091	4.35E-06	JHS
X:154533025	A/G	667	0.004	5.24	-0.484	0.130	2.01E-04	MESA
X:154533025	A/G	3,123	0.005	32.85	-0.436	0.073	2.16E-09	JHS+MESA+ARIC

^aMarkerID defined as Chromosome:Position. Positions are indicated on Build 38.

^bEAf: Effect Allele Frequency

^cAC: Allele Count

Supplemental Methods

Cohort Description

1. Cohorts included in the HbA1c and RBC analyses

The Amish study

The Amish Complex Disease Research Program (<http://www.medschool.umaryland.edu/endocrinology/Amish-Research-Program/>) includes a set of large community-based studies focused largely on cardiometabolic health carried out in the Old Order Amish (OOA) community of Lancaster County, Pennsylvania. Over 7,000 Amish have been recruited to date. This Amish community is a founder population who immigrated to Pennsylvania from Western Europe in the early 1700's, later expanding into other regions of the U.S. The Amish cohort participating in the TOPMed Consortium comprises 1,120 subjects ≥ 18 years of age from large multigenerational families who were recruited for specific protocols between 2001 and 2006. Subjects have been extensively phenotyped for a range of cardiometabolic traits, including anthropometry, lipids, blood pressure, glucose and related measures, vascular imaging, and a range of other phenotypes. DNA samples have been collected and serum and plasma samples biobanked. The TOPMed Program has provided WGS data to complement GWAS array data already collected in >5,000 Amish study participants. Due to their ancestral history, the OOA are enriched for rare exonic variants that arose in the population from a single founder (or small number of founders) and propagated through genetic drift. Many of these variants have large effect sizes, and identifying them can lead to new biological insights about health and disease. A major goal of the TOPMed WGS sequencing efforts is to identify functional variants that underlie some of the large effect associations observed in this unique population.

Complete Blood Counts analyses were performed within 24 h for all individuals and consisted of RBC, HB, HCT, MCV, MCH, MCHC, RDW and WBC. RBC, HB, and MCV were directly measured, whereas HCT, MCH, and MCHC were mathematically derived from directly measured erythrocyte traits ($MCV \times RBC$, HB/RBC , and MCH/MCV , respectively).¹

The Atherosclerosis Risk in Communities Study

The ARIC study is a population-based prospective cohort study of cardiovascular disease sponsored by the National Heart, Lung, and Blood Institute (NHLBI). ARIC included 15,792 individuals, predominantly European American and African American, aged 45-64 years at baseline (1987-89) and chosen by probability sampling from four US communities. Cohort members completed three additional triennial follow-up examinations, a fifth exam in 2011-2013, and a sixth exam in 2016-2017. The ARIC study has been described in detail previously.² Complete Blood Counts was measured using automated hematology analyzers: Coulter S + IV (calibration S - Cal, Beckman Coulter, Inc, Fullerton, CA) at 2 sites, Coulter S + III and Coulter S + IV (calibration S- Cal) at 1 site, and Technicon H-6000 (calibration Fisher, Technicon Corporation, Tarrytown, NY) at 1 site.

The Framingham Heart Study

The Framingham Heart Study (FHS) is a single-site, community-based, prospective cohort study that was initiated in 1948 to investigate risk factors for cardiovascular disease. The population of Framingham was almost entirely white in 1948. The FHS comprises three

generations of participants: the original cohort followed since 1948 (Original or Gen1);³ their offspring and spouses of the offspring, followed since 1971 (Offspring or Gen2);⁴ and children from the largest offspring families enrolled in 2002 (Gen3).⁵ The Original cohort enrolled 5,209 men and women who comprised two-thirds of the adult population then residing in Framingham, MA, USA. Survivors continue to receive biennial examinations. The Offspring cohort comprises 5,124 persons (including 3,514 biological offspring) who have been examined approximately once every 4 to 8 years and have completed 9 examinations. The first examination of the Gen3 was completed in July 2005 and included 4,095 participants, a third examination is currently ongoing. All cohorts continue under active surveillance for cardiovascular events. All participants provided written informed consent. This study was approved by the Institutional Review Board of the Boston University Medical Center.

The determination of hematological phenotypes in the FHS has been detailed previously.^{6,7} Hematology testing was performed on a Baker 9000 Hematology Analyzer (Baker Instruments Corp.) for the Original cohort and on a Beckman Coulter HmX Hematology Analyzer (Beckman Coulter, Inc.) for the Offspring and Gen3 cohorts.

The Jackson Heart Study

The Jackson Heart Study (JHS) was designed to study the reasons for the greater prevalence of cardiovascular disease among African Americans and to find new approaches for reducing this health disparity. JHS recruited 5,306 AA participants from urban and rural areas of the three counties (Hinds, Madison and Rankin) that comprise the Jackson, Mississippi metropolitan area from 2000-2004. Recruitment was limited to non-institutionalized adult African Americans. Participants were recruited in four ways: (1) randomly sampling households from a commercial listing; (2) a structured volunteer sample designed to mirror the eligible population; (3) current enrollment in the Atherosclerosis Risk in Communities (ARIC) study; and (4) a nested family cohort. Unrelated participants were between 35 and 84 years old, while members of the family cohort were ≥ 21 years old at baseline. A range of measures, including health behaviors, medication use, anthropometry, blood pressure, assessments of kidney function and diabetes, and CVD biomarkers, were assessed at the baseline JHS visit. The JHS has been described in detail previously.^{8,9} Blood cell count metrics were assessed using standard methods (Beckman Coulter automated hematology analyzer), as described previously.¹⁰

The Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA consisted of a diverse, population-based sample of an initial 6,814 asymptomatic men and women aged 45-84. 38 percent of the recruited participants were white, 28 percent African American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent. Participants were recruited from six field centers across the United States: Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California - Los Angeles. Each participant received an extensive physical exam and determination of coronary calcification, ventricular mass and function, flow-mediated endothelial vasodilation, carotid intimal-medial wall thickness and presence of echogenic lucencies in the carotid artery, lower extremity vascular insufficiency,

arterial wave forms, electrocardiographic (ECG) measures, standard coronary risk factors, sociodemographic factors, lifestyle factors, and psychosocial factors. Selected repetition of subclinical disease measures and risk factors at follow-up visits allowed study of the progression of disease. Participants are being followed for identification and characterization of cardiovascular disease events, including acute myocardial infarction and other forms of coronary heart disease (CHD), stroke, and congestive heart failure; for cardiovascular disease interventions; and for mortality. The first examination took place over two years, from July 2000 - July 2002. It was followed by four examination periods that were 17-20 months in length. Participants have been contacted every 9 to 12 months throughout the study to assess clinical morbidity and mortality.

MESA Family

In the MESA Family Study, the goal is to locate and identify genes contributing to the genetic risk for cardiovascular disease (CVD), by looking at the early changes of atherosclerosis within families (mainly siblings). 2128 individuals from 594 families, yielding 3,026 sib pairs divided between African Americans and Hispanic-Americans, were recruited by utilizing the existing framework of MESA. MESA Family studied siblings of index subjects from the MESA study and from new sibpair families (with the same demographic characteristics) and is determining the extent of genetic contribution to the variation in coronary calcium (obtained via CT Scan) and carotid artery wall thickness (B-mode ultrasound) in the two largest non-majority U.S. populations. The MESA Family cohort was recruited from the six MESA Field Centers. MESA Family participants underwent the same examination as MESA participants during May 2004 - May 2007. The MESA Study has been described in detail previously.¹¹ Complete Blood Counts was performed at LabCorp using automated cell counter with mixed technologies.

2. Cohorts included in the RBC analyses only

The Cardiovascular Health Study

CHS is a population-based cohort study of risk factors for coronary heart disease and stroke in adults ≥ 65 years conducted across four field centers.¹² The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled for a total sample of 5,888. CHS participants selected for inclusion in the TOPMed sequencing program included African-Americans participants, cases of idiopathic venous thromboembolism, myocardial infarction, coronary heart disease or stroke and a random sample of "healthy elderly". Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. Details of measurement of hematologic traits have been previously described.¹³

CHS was approved by institutional review committees at each field center and individuals in the present analysis had available DNA and gave informed consent including consent to use of genetic information for the study of cardiovascular disease.

The Genetic Studies of Atherosclerosis Risk

GeneSTAR: The Genetic Study of Atherosclerosis Risk (GeneSTAR) is a longitudinal family-based study designed to explore environmental, phenotypic, and genetic causes of premature cardiovascular disease. European- and African-American participants were

recruited from European- and African-American families (n=891) identified from probands hospitalized for a coronary disease event prior to 60 years of age in any of 10 Baltimore, Maryland area hospitals between 1983 and 2006. Apparently healthy siblings of the probands, offspring of the siblings and probands, and the co-parents of the offspring were screened for traditional coronary disease and stroke risk factors between 2003 and 2006 as part of a platelet function study of two weeks of 81 mg/day of aspirin.^{14, 15} All measures described here were obtained prior to the commencement of aspirin. Exclusion criteria included: 1) any coronary heart disease or vascular thrombotic event, 2) any bleeding disorder or hemorrhagic event, 3) current use of any anticoagulants or anti-platelet agents, 4) current use of chronic or acute nonsteroidal anti-inflammatory agents that could not be discontinued, 5) recent active gastrointestinal disorder, 6) current pharmacotherapy for a gastrointestinal disorder, 7) pregnancy or risk of pregnancy during the trial, 8) recent menorrhagia, 9) known aspirin intolerance or allergic side effects, 10) serious medical disorders, (eg, autoimmune diseases, cancer or HIV-AIDS), 11) current chronic or acute use of glucocorticosteroid therapy or any drug that may interfere with the measured outcomes, 12) serious psychiatric disorders, and, 13) inability to independently make a decision to participate. Of 3003 aspirin study participants, 1786 were selected for TOPMed based on 1) complete platelet function phenotyping and 2) largest family size. Complete Cell Counts were obtained using an automated cell counter (ACT-Diff, Beckman Coulter).

The San Antonio Family Study

The San Antonio Family Study results from the amalgamation of two San Antonio-based genetic studies. The first is the longitudinal San Antonio Family Heart Study (SAFHS) which began in 1991 and was designed to primarily investigate the genetics of cardiovascular disease and its risk factors in Mexican Americans. The SAFHS included 1,431 individuals in 42 extended families at baseline.¹⁶ With some additional recruiting, it has now been expanded to 1,662 individuals in 47 families. Ascertainment occurred by way of the random selection of an adult Mexican American proband, without regard to presence or absence of disease and almost exclusively from Mexican American census tracts in San Antonio. The second component study is the San Antonio Family Gall Bladder Study (Dr. Duggirala, PI) which included 907 individuals from 39 families ascertained similarly to the SAFHS but with the requirement that the original proband also be diabetic.¹⁷ This is a very weak form of ascertainment in Mexican Americans, where lifetime prevalence of diabetes approaches 30%. In fact, 20 years after the initiation of the SAFHS, the prevalence for major diseases such as heart disease, diabetes, and obesity are not significantly different between these two component studies. Finally, we have expanded these pedigrees in recent years by examining a set of 498 children than are part of these families. This expansion was part of Dr. Duggirala's San Antonio Family Assessment of Metabolic Risk Factors in Youth (SAFARI) study. Additionally, we have seen 112 of these children subsequently as adults. Combined, these studies have 3,099 individuals primarily from 73 families. Our study is a mixed longitudinal design. Subjects have been seen between 1 and 4 times with an average of 1.95 examinations. For this study, samples and data analyzed were from the San Antonio Family Heart Study component. Blood cell counts were obtained within 2hrs of blood collection from an EDTA tube using a Beckman Coulter AcT diff2 Hematology Analyzer.

The Women's Health Initiative

The Women's Health Initiative (WHI) is a long-term, prospective, multi-center cohort study investigating post-menopausal women's health in the US.¹⁸ WHI was funded by the National Institutes of Health and the National Heart, Lung, and Blood Institute to study strategies to prevent heart disease, breast cancer, colon cancer, and osteoporotic fractures in women 50-79 years of age. WHI involves 161,808 women recruited between 1993 and 1998 at 40 centers across the US. The study consists of two parts: the WHI Clinical Trial which was a randomized clinical trial of hormone therapy, dietary modification, and calcium/Vitamin D supplementation, and the WHI Observational Study, which focused on many of the inequities in women's health research and provided practical information about incidence, risk factors, and interventions related to heart disease, cancer, and osteoporotic fractures. Fasting blood samples were drawn and analyzed for RBC traits at designated clinical laboratories using an automated electronic cell counter at the baseline examination.¹⁹ Participants with $HCT > 80$, $HGB > 30$, $RBC = 0$ or $HCT/HGB > 7$ were excluded. In total, 9912, 9909 and 1285 participants were included for HGB, HCT and RDW analyses, respectively, and 1286 participants were included for MCH, MCHC, MCV and RBC counts analyses.

Supplemental Acknowledgements

Whole genome sequencing:

WGS for "NHLBI TOPMed: Genetics of Cardiometabolic Health in the Amish (phs000956.v1.p1) was performed at the Broad Institute of MIT and Harvard (3R01HL121007-01S1). WGS for "NHLBI TOPMed: Trans-Omics for Precision Medicine Whole Genome Sequencing Project: ARIC (phs001211.v1.p1) was performed at the Broad Institute of MIT and Harvard and at the Baylor Human Genome Sequencing Center (3R01HL092577-06S1 (Broad, AFGen), HHSN268201500015C (Baylor, VTE), 3U54HG003273-12S2 (Baylor, VTE)). WGS for "NHLBI TOPMed: Whole Genome Sequencing and Related Phenotypes in the Framingham Heart Study (phs000974.v1.p1) was performed at the Broad Institute of MIT and Harvard (3R01HL092577-06S1 (AFGen)). WGS for "NHLBI TOPMed: The Jackson Heart Study (phs000964.v1.p1) was performed at the University of Washington Northwest Genomics Center (HHSN268201100037C). WGS for "NHLBI TOPMed: MESA and MESA Family AA-CAC (phs001416) was performed at the Broad Institute of MIT and Harvard (3U54HG003067-13S1 (MESA, TOPMed supplement to NHGRI), HHSN268201500014C (Broad, AA_CAC)). WGS for "NHLBI TOPMed: Cardiovascular Health Study (phs001368.v1.p1)" was performed at Baylor Human Genome Sequencing Center (HHSN268201500015C, VTE portion of CHS). WGS for "NHLBI TOPMed: GeneSTAR (Genetic Study of Atherosclerosis Risk) (phs001218.v1.p1)" was performed at MacroGen Corp and at the Broad Institute of MIT and Harvard (HHSN268201500014C (Broad, AA_CAC)). WGS for "NHLBI TOPMed: Women's Health Initiative (WHI) (phs001237.v1.p1)" was performed at the Broad Institute of MIT and Harvard (HHSN268201500014C). WGS for "NHLBI TOPMed: San Antonio Family Heart Study (WGS) (phs001215.v1.p1)" was performed at Illumina Genomic Services (3R01HL113323-03S1).

The Amish study

NIH grant R01 HL121007

The Atherosclerosis Risk in Communities Study

Whole genome sequencing (WGS) for the Trans-Omics in Precision Medicine (TOPMed) program was supported by the National Heart, Lung and Blood Institute (NHLBI). WGS for "NHLBI TOPMed: Atherosclerosis Risk in Communities (ARIC)" (phs001211) was performed at the Baylor College of Medicine Human Genome Sequencing Center (HHSN268201500015C and 3U54HG003273-12S2) and the Broad Institute for MIT and Harvard (3R01HL092577-06S1). Centralized read mapping and genotype calling, along with variant quality metrics and filtering were provided by the TOPMed Informatics Research Center (3R01HL-117626-02S1). Phenotype harmonization, data management, sample-identity QC, and general study coordination, were provided by the TOPMed Data Coordinating Center (3R01HL-120393-02S1). We gratefully acknowledge the studies and participants who provided biological samples and data for TOPMed.

The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services (contract numbers HHSN268201700001I,

HHSN268201700002I, HHSN268201700003I, HHSN268201700004I and HHSN268201700005I). The authors thank the staff and participants of the ARIC study for their important contributions.

The Cardiovascular Health Study

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants R01HL120393, U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

The Framingham Heart Study

This research was conducted in part using data and resources from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The Framingham Heart Study (FHS) acknowledges the support of contracts NO1-HC-25195 and HHSN268201500001I from the National Heart, Lung and Blood Institute, contract 75N92019D00031 and grant supplement R01 HL092577-06S1 for this research. This work was also supported in part by grant U01DK078616.

We also acknowledge the dedication of the FHS study participants without whom this research would not be possible.

The Genetic Studies of Atherosclerosis Risk

GeneSTAR was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute (U01 HL72518, HL087698, HL112064, HL11006, HL118356) and by a grant from the National Institutes of Health/National Center for Research Resources (M01-RR000052) to the Johns Hopkins General Clinical Research Center. We would like to thank the participants and families of GeneSTAR and our dedicated staff for all their sacrifices.

The Jackson Heart Study

The Jackson Heart Study (JHS) is supported and conducted in collaboration with Jackson State University (HHSN268201800013I), Tougaloo College (HHSN268201800014I), the Mississippi State Department of Health (HHSN268201800015I/HHSN26800001) and the University of Mississippi Medical Center (HHSN268201800010I, HHSN268201800011I and HHSN268201800012I) contracts from the National Heart, Lung, and Blood Institute (NHLBI) and the National Institute for Minority Health and Health Disparities (NIMHD). The authors also wish to thank the staffs and participants of the JHS.

The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

The Multi-Ethnic Study of Atherosclerosis

Whole genome sequencing (WGS) for the Trans-Omics in Precision Medicine (TOPMed) program was supported by the National Heart, Lung and Blood Institute (NHLBI). WGS for “NHLBI TOPMed: Multi-Ethnic Study of Atherosclerosis (MESA)” (phs001416.v1.p1)

was performed at the Broad Institute of MIT and Harvard (3U54HG003067-13S1). Centralized read mapping and genotype calling, along with variant quality metrics and filtering were provided by the TOPMed Informatics Research Center (3R01HL-117626-02S1). Phenotype harmonization, data management, sample-identity QC, and general study coordination, were provided by the TOPMed Data Coordinating Center (3R01HL-120393-02S1). MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-000040, UL1-TR-001079, UL1-TR-001420. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR001881, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

The San Antonio Family Study

Collection of the San Antonio Family Study data was supported in part by National Institutes of Health (NIH) grants R01 HL045522, DK047482, DK053889, MH078143, MH078111 and MH083824; and whole genome sequencing of SAFS subjects was supported by U01 DK085524 and R01 HL113323. This work was conducted in part in facilities constructed under the support of NIH grant C06 RR020547. We are very grateful to the participants of the San Antonio Family Study for their continued involvement in our research programs.

The Women's Health Initiative

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: <http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf>

The UK Biobank

This research has been conducted using the UK Biobank Resource under Application Number 42614.

References

1. Hinckley, J.D., Abbott, D., Burns, T.L., Heiman, M., Shapiro, A.D., Wang, K., Di Paola, J. (2013). Quantitative trait locus linkage analysis in a large Amish pedigree identifies novel candidate loci for erythrocyte traits. *Mol. Genet. Genomic Med.* *1*, 131-141.
2. Anonymous (1989). The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am. J. Epidemiol.* *129*, 687-702.
3. Dawber, T.R., Kannel, W.B. (1966). The Framingham study. An epidemiological approach to coronary heart disease. *Circulation* *34*, 553-555.
4. Feinleib, M., Kannel, W.B., Garrison, R.J., McNamara, P.M., Castelli, W.P. (1975). The Framingham Offspring Study. Design and preliminary data. *Prev. Med.* *4*, 518-525.
5. Splansky, G.L., Corey, D., Yang, Q., Atwood, L.D., Cupples, L.A., Benjamin, E.J., D'Agostino RB, S., Fox, C.S., Larson, M.G., Murabito, J.M. et al. (2007). The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am. J. Epidemiol.* *165*, 1328-1335.
6. Yang, Q., Kathiresan, S., Lin, J.P., Tofler, G.H., O'Donnell, C.J. (2007). Genome-wide association and linkage analyses of hemostatic factors and hematological phenotypes in the Framingham Heart Study. *BMC Med. Genet.* *8 Suppl 1*, S12-2350-8-S1-S12.
7. Sloan, A., Gona, P., Johnson, A.D. (2015). Cardiovascular correlates of platelet count and volume in the Framingham Heart Study. *Ann. Epidemiol.* *25*, 492-498.
8. Taylor, H.A., Jr, Wilson, J.G., Jones, D.W., Sarpong, D.F., Srinivasan, A., Garrison, R.J., Nelson, C., Wyatt, S.B. (2005). Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study. *Ethn. Dis.* *15*, S6-4-17.
9. Wilson, J.G., Rotimi, C.N., Ekunwe, L., Royal, C.D., Crump, M.E., Wyatt, S.B., Steffes, M.W., Adeyemo, A., Zhou, J., Taylor, H.A., Jr et al. (2005). Study design for genetic analysis in the Jackson Heart Study. *Ethn. Dis.* *15*, S6-30-37.
10. Carpenter, M.A., Crow, R., Steffes, M., Rock, W., Heilbraun, J., Evans, G., Skelton, T., Jensen, R., Sarpong, D. (2004). Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study. *Am. J. Med. Sci.* *328*, 131-144.
11. Bild, D.E., Bluemke, D.A., Burke, G.L., Detrano, R., Diez Roux, A.V., Folsom, A.R., Greenland, P., Jacob, D.R., Jr, Kronmal, R., Liu, K. et al. (2002). Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am. J. Epidemiol.* *156*, 871-881.
12. Fried, L.P., Borhani, N.O., Enright, P., Furberg, C.D., Gardin, J.M., Kronmal, R.A., Kuller, L.H., Manolio, T.A., Mittelmark, M.B., Newman, A. (1991). The Cardiovascular Health Study: design and rationale. *Ann. Epidemiol.* *1*, 263-276.
13. Ganesh, S.K., Zakai, N.A., van Rooij, F.J., Soranzo, N., Smith, A.V., Nalls, M.A., Chen, M.H., Kottgen, A., Glazer, N.L., Dehghan, A. et al. (2009). Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. *Nat. Genet.* *41*, 1191-1198.
14. Bray, P.F., Mathias, R.A., Faraday, N., Yanek, L.R., Fallin, M.D., Herrera-Galeano, J.E., Wilson, A.F., Becker, L.C., Becker, D.M. (2007). Heritability of platelet function in families with premature coronary artery disease. *J. Thromb. Haemost.* *5*, 1617-1623.

15. Faraday, N., Yanek, L.R., Mathias, R., Herrera-Galeano, J.E., Vaidya, D., Moy, T.F., Fallin, M.D., Wilson, A.F., Bray, P.F., Becker, L.C. et al. (2007). Heritability of platelet responsiveness to aspirin in activation pathways directly and indirectly related to cyclooxygenase-1. *Circulation* *115*, 2490-2496.
16. Mitchell, B.D., Kammerer, C.M., Blangero, J., Mahaney, M.C., Rainwater, D.L., Dyke, B., Hixson, J.E., Henkel, R.D., Sharp, R.M., Comuzzie, A.G. et al. (1996). Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. *Circulation* *94*, 2159-2170.
17. Duggirala, R., Blangero, J., Almasy, L., Dyer, T.D., Williams, K.L., Leach, R.J., O'Connell, P., Stern, M.P. (1999). Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am. J. Hum. Genet.* *64*, 1127-1140.
18. Anonymous (1998). Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control. Clin. Trials* *19*, 61-109.
19. Chen, Z., Tang, H., Qayyum, R., Schick, U.M., Nalls, M.A., Handsaker, R., Li, J., Lu, Y., Yanek, L.R., Keating, B. et al. (2013). Genome-wide association analysis of red blood cell traits in African Americans: the COGENT Network. *Hum. Mol. Genet.* *22*, 2529-2538.