Hemoglobin A_{1c} Genetics and Disparities in Risk of Diabetic Retinopathy in Individuals of Genetically Inferred African American/African British and European Ancestries

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OBJECTIVE

Individuals with diabetes who carry genetic variants that lower hemoglobin A_{1c} (HbA_{1c}) independently of glycemia may have higher real, but undetected, hyperglycemia compared with those without these variants despite achieving similar HbA_{1c} targets, potentially placing them at greater risk for diabetes-related complications. We sought to determine whether these genetic variants, aggregated in a polygenic score, and the large-effect African ancestry–specific missense variant in *G6PD* (rs1050828) that lower HbA_{1c} were associated with higher retinopathy risk.

RESEARCH DESIGN AND METHODS

Using data from 29,828 type 2 diabetes cases of genetically inferred African American/African British and European ancestries, we calculated ancestry-specific nonglycemic HbA_{1c} polygenic scores (ngA1cPS) composed of 122 variants associated with HbA_{1c} at genome-wide significance, but not with glucose. We tested the association of the ngA1cPS and the *G6PD* variant with retinopathy, adjusting for measured HbA_{1c} and retinopathy risk factors.

RESULTS

Participants in the bottom quintile of the ngA1cPS showed between 20% and 50% higher retinopathy prevalence, compared with those above this quintile, despite similar levels of measured HbA_{1c}. The adjusted meta-analytic odds ratio for the bottom quintile was 1.31 (95% CI 1.0, 1.73; $P = 0.05$) in African ancestry and 1.31 (95% CI 1.15, 1.50; $P = 6.5 \times 10^{-5}$) in European ancestry. Among individuals of African ancestry with HbA_{1c} below 7%, retinopathy prevalence was higher in individuals below, compared with above, the 50th percentile of the ngA1cPS regardless of sex or *G6PD* carrier status.

CONCLUSIONS

Genetic effects need to be considered to personalize HbA_{1c} targets and improve outcomes of people with diabetes from diverse ancestries.

The Precision Medicine Initiative aims to enable a new era of medicine through research, technology, and policies to develop individualized care. Yet, it remains unclear

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how genetic information can be used in routine diabetes care (1,2). One application of precision medicine is to account for genetic variation that influences the performance of hemoglobin A_{1c} (HbA_{1c}), a widely used biomarker that measures the proportion of glycated hemoglobin to estimate ambient glycemia over the preceding 2–3 months. HbA_{1c} is a key modifiable risk factor for both macro- and microvascular complications (3–6) and has been accepted as the preferred diagnostic test for diabetes and measure of glycemic control, and as a clinical tool for managing complication risks.

Genetic variation that influences HbA_{1c} through nonglycemic mechanisms (e.g., differences in erythrocyte life span) can affect how accurately HbA_{1c} reflects underlying glycemia (7,8). In a large-scale, multiancestry genome-wide association study (GWAS) meta-analysis of HbA_{1c}, more than 200 genetic variants were reported to be associated with HbA_{1c} (9–15). The effect of being in the top 5%, relative to the bottom 5%, of a polygenic score comprising 60 genetic variants that influence HbA_{1c} independently of glycemia was 0.25% in individuals of European ancestry, but 0.8% in individuals of African ancestry (13,16). This large ancestral difference was due to a single African-specific missense variant in *G6PD*, rs1050828 (risk allele, T), which has a minor allele frequency of ~12% in African Americans, lowers HbA_{1c} independently of glycemia, and causes glucose-6-phosphatase dehydrogenase (G6PD) deficiency, an X-linked disease (17,18).

Clinical practice guidelines have chosen the HbA_{1c} diagnostic threshold of 6.5% and the HbA_{1c} target of 7% for most adults, due to their associations with the risk of developing diabetes-related complications (19,20). However, people who carry genetic variants that lower HbA_{1c} independently of glycemia may be delayed in their diabetes diagnosis and undertreated for hyperglycemia, creating disparities in outcomes, especially among certain minority populations with a higher prevalence of large-effect variants, like the African-specific *G6PD* variant.

The objective of this study was to evaluate the nature and extent of such disparities by evaluating the effects of genetically driven nonglycemic variation in HbA_{1c} on the risk of retinopathy in people with type 2 diabetes. We hypothesized that individuals who carry genetic variants that lower HbA_{1c} independently of glycemia have higher real, but undetected, hyperglycemia compared with others who do not and, consequently, have a greater risk of developing diabetes-related complications, despite achieving similar HbA_{1c} targets. We chose retinopathy as the outcome because it is one of the earliest complications of diabetes and can develop even before a diabetes diagnosis if chronic hyperglycemia was undetected (6,21,22). We tested this hypothesis using data from 29,828 individuals with type 2 diabetes in the UK Biobank (UKBB) and All of Us (AoU) databases with genetically inferred African African/African British or European ancestry. We calculated ancestry-specific nonglycemic HbA_{1c} polygenic scores (ngA1cPS) using a weighted sum of genetic variants previously associated with HbA_{1c} in GWAS, but not with glucose. We evaluated whether individuals with a low ngA1cPS, indicating a genetic predisposition to lower HbA_{1c} independently of glycemia, had a greater risk of retinopathy, adjusting for measured HbA_{1c} and established retinopathy risk factors. In doing so, we evaluated disparities in retinopathy prevalence across the ngA1cPS and by ancestry to determine whether accounting for genetic effects could improve the clinical utility of HbA_{1c} in diverse populations or explain interindividual variation in outcomes despite seemingly achieving HbA_{1c} targets.

RESEARCH DESIGN AND METHODS

Overview of Cohorts and Genetic Data

The UKBB is a prospective cohort study with genetic and phenotypic data collected on approximately 500,000 individuals from the U.K. who were between 40 and 69 years of age at recruitment (23). Using the UKBB array data, we applied preimputation quality control, performed phasing with SHAPEIT4 (https://odelaneau.github.io/shapeit4/), and imputed the phased haplotypes using the TOPMed reference panel freeze 8 (24).

The AoU Research Program is a U.S. biobank developed to leverage the diversity of the United States for facilitating and improving high powered genetic and epidemiological studies. Details of the recruitment methods, clinical sites, and data availability are described elsewhere (25). On 22 June 2022, the AoU Research Program released whole-genome sequencing data for 98,590 participants. Electronic health records and genetic information were extracted from the AoU, version 6, controlled tier data set using the AoU Researcher Workbench.

Outcomes and Variables

Type 2 diabetes, retinopathy, coronary artery disease, chronic kidney disease, and hypertension were defined using the ICD-9 and -10 codes, shown in Supplementary Table 1. Biological sex was inferred from genetic data. Diabetes duration was calculated as the number of years between the patient's self-reported age of diagnosis and age at the time of enrollment. We used the mean value calculated across available measurements for diastolic and systolic blood pressure, HbA_{1c}, random glucose, triglycerides, LDL, and creatinine. Individuals with missing HbA_{1c} data were excluded from the analyses. For the remaining laboratory values, the mean missing rate was 7% (median, 8%), and missing values were imputed with the median of the nonmissing values (Supplementary Table 2).

Construction of the ngA1cPS by Ancestry

For individuals of European ancestry, we calculated an ngA1cPS composed of 122 variants reported to be associated with HbA_{1c} at genome-wide significance in a multiancestry meta-analysis GWAS in people without diabetes by the Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) that had less than 25% probability of being assigned "glycemic" based on their association with glycemic traits, red blood cell traits, and iron metabolism in a soft clustering analysis (15). The threshold of 25% was selected to achieve a balance between attaining enough variants to form a polygenic score that explains genetically driven HbA_{1c} variation while excluding variants clearly associated with glycemic traits. In European ancestry, of the 185 HbA_{1c} variants, 63 were above this threshold due to their association with glycemia and were excluded, leaving 122 variants to construct the ngA1cPS (Supplementary Table 3). The ngA1cPS was applied with the score function in PLINK (https://www.cog-genomics.org/plink/1.9/), using the β values from the original GWAS as the weights with the effect allele defined as the HbA_{1c}-raising allele (i.e., all weights used in the ngA1cPS are positive; Supplementary Table 4). Because only 14 of the 122 variants discovered in

the multiancestry GWAS meta-analysis were associated with HbA_{1c} at nominal significance in the African-ancestry GWAS (15), we tested the association of an ngA1cPS composed of only the 14 variants with HbA_{1c} in our study sample and found the association to be stronger than the 122-variant ngA1cPS in African ancestry (Supplementary Table 5). Thus, we opted to use the 14-variant ngA1cPS in the analysis of African ancestry. Because the effect of the *G6PD* variant on HbA_{1c} had a reported effect size that was many times larger than all the other variants combined (13), we excluded it from the ngA1cPS and examined the effect of the *G6PD* variant separately.

Logistic Regression and Retinopathy Prevalence by Ancestry, ngA1cPS Quintiles, HbA_{1c} Categories, and *G6PD* Carrier Status

We plotted the proportion of retinopathy by ngA1cPS quintiles and by ancestry to visually inspect for differences. We fitted a logistic regression model to assess the effect being in the bottom quintile (ngA1cPS percentile <20), compared with the middle 60%, on retinopathy, and adjusted for age, genetic sex, and 10 genetic ancestry principal components, followed by measured HbA_{1c} and other retinopathy risk factors, including diabetes duration, chronic kidney disease, hypertension; random glucose, triglyceride, LDL, and creatinine levels; and current smoking. We calculated the effects in UKBB and AoU separately and then performed a fixed-effects meta-analysis within each ancestry. To determine whether the study had sufficient statistical power to detect meaningful effects, we performed a power calculation and showed that, in a case-control study with 498 cases in African American/African British ancestry and 1,862 cases in European ancestry and an equal number of controls, using α = 0.025, we had at least 80% power to detect an odds ratio (OR) as small as 1.20 in African American/African British ancestry and 1.10 in European ancestry per SD of the ngA1cPS. Due to the smaller sample of African American/African British ancestry, we acknowledge that subtle associations could be missed.

In European ancestry, we performed stratified analyses by measured HbA_{1c} categories of 0.5%. In African ancestry, due to the small number of *G6PD* carriers with retinopathy, particularly in the UKBB database, we restricted the stratified analysis by *G6PD* carrier status to AoU and compared individuals below the 50th percentile with those above the 50th percentile of the ngA1cPS instead of quintiles. Because the *G6PD* variant is located on the X chromosome, we reported results stratified by sex. The Mass General Brigham Institutional Review Board (study no. 2016P001018) approved this study. All participants in UKBB (National Research Ethics Committee reference no. 11/NW/0382) and AoU provided informed consent, and research was conducted according to the Declaration of Helsinki.

RESULTS

Population Characteristics

In the UKBB and AoU, we identified 25,895 and 11,165 individuals with type 2 diabetes, of whom 1,471 and 1,639, respectively, had retinopathy (Table 1). Of these 37,060 individuals, 32,350 were of European or African ancestry, and 29,828 had available HbA_{1c} data for downstream analysis. In both cohorts, individuals with retinopathy were slightly older (62 vs. 61 years, $P = 3\times10^{-19}$ in UKBB; 65 vs. 63 years old, $P = 3\times10^{-10}$ in AoU) and had longer diabetes duration (13.4 vs. 7.5 years, $P = 5\times10^{-141}$ in UKBB; 11.0 vs. 7.5 years, $P = 9\times10^{-146}$ in AoU). Individuals with retinopathy had higher blood pressure, higher HbA_{1c} (7.7% vs. 7.0%, $P = 1\times10^{-98}$ in UKBB; 8.0% vs. 7.0%, $P = 4\times10^{-86}$ in AoU), higher random glucose levels, and higher proportions of chronic kidney disease, coronary artery disease, and hypertension. In both populations, we observed a smaller proportion of females with retinopathy compared with those without (32% vs. 38%, $P = 1\times10^{-5}$ in UKBB; 50.0% vs. 58.7%, $P = 7\times10^{-9}$ in AoU).

Retinopathy Prevalence Across the ngA1cPS

In both African American/African British and European ancestries, the bottom quintile of the ngA1cPS had the lowest retinopathy prevalence (Fig. 1*A* and *B*). The median measured HbA_{1c} in the bottom quintile, middle three quintiles, and top quintile of the ngA1cPS were 6.39% (interquartile range [IQR] = 5.79, 7.29), 6.47% (IQR = 5.9, 7.38), and 6.52% (IQR = 5.99, 7.30), and the corresponding median random glucose levels were 109.0 mg/dL (IQR = 91.4, 146.6), 107.9 mg/dL (IQR = 90.9, 143.1), 106.3 mg/dL (IQR = 90.6, 140.4), respectively. Creatinine, hemoglobin, reticulocyte percentage, and the proportion of individuals reported to be on metformin and insulin were similar across the ngA1cPS for both ancestries (Supplementary Table 6).

Association of the ngA1cPS With Prevalent Retinopathy

The distributions of the ngA1cPS by retinopathy status overlapped, indicating that the ngA1cPS cannot discriminate those with and without retinopathy, though the means of the ngA1cPS were lower in people with retinopathy compared with those without (Supplementary Fig. 1). Despite this lack of discrimination, the ngA1cPS was associated with retinopathy even after adjustment for measured HbA_{1c} and other retinopathy risk factors in the meta-analysis of UKBB and AoU. The meta-analytic OR for prevalent retinopathy in the bottom quintile of the ngA1cPS, adjusted for measured HbA_{1c}, was 1.45 (95% CI 1.13, 1.88; $P = 3.5\times10^{-3}$) in African American/African British ancestry (Fig. 1*C*) and 1.34 (95% CI 1.19, 1.51; $P = 9.8\times10^{-7}$) in European ancestry (Fig. 1*D*). The meta-analytic ORs after further adjusting for other retinopathy risk factors was 1.31 (95% CI 0.1.0, 1.73; $P = 0.05$) in African American/African British ancestry and 1.31 (95% CI 1.15, 1.50; $P = 6.5\times10^{-5}$) in European ancestry.

In the analysis stratified by measured HbA_{1c} categories in European ancestry, the meta-analytic ORs, adjusted for age, sex, and principal components and retinopathy risk factors, were largest in individuals with HbA_{1c} between 6% and 6.5% (OR 1.48; 95% CI 1.05, 2.09; $P = 0.02$; Fig. 2*A* and *B*), and HbA_{1c} between 7.5% and 8% (OR 1.59; 95% CI 1.07, 2.38; $P = 0.02$; Fig. 2*A* and 2*B*) The ngA1cPS was marginally associated with prevalent retinopathy in individuals with HbA_{1c} above 8% and below 6%.

There were no statistically significant differences in retinopathy prevalence by *G6PD* genotype and the median measured HbA_{1c} was similar across *G6PD* genotypes (6.8% in noncarriers; 6.9% in heterozygous females; 6.7% in affected males and homozygous female). However, among individuals with HbA_{1c} below 7%, retinopathy prevalence appeared to be higher in *G6PD* carriers compared with

Table 1—Characteristics of participants with type 2 diabetes in UKBB and AoU by retinopathy diagnosis[a]

	UKBB			AoU		
Characteristic	Retinopathy (n = 1,471)	No retinopathy (n = 24,424)	P	Retinopathy (n = 1,639)	No retinopathy (n = 9,526)	P
Age, mean ± SD, years	62.3 ± 6.3	60.6 ± 7.1	2.93×10^{-19}	64.9 ± 12.5	62.7 ± 13.2	3.24×10^{-10}
Female, n (%)	469 (31.9)	9176 (37.6)	1.34×10^{-5}	820 (50.0)	5,501 (58.7)	6.81×10^{-9}
Genetic ancestry, n (%)						
African	74 (5.0)	965 (4.0)	0.01	424 (25.9)	2,443 (25.6)	1.31×10^{-9}
Amerindian/Latin American	3 (0.2)	54 (0.2)		390 (23.8)	1,724 (18.1)	
South Asian	140 (9.5)	1,810 (7.4)		<20	78 (0.8)	
East Asian	6 (0.4)	181 (0.7)		36 (2.2)	111 (1.2)	
European	1,230 (83.6)	21,125 (86.5)		632 (38.6)	4,338 (45.5)	
Middle Eastern	9 (0.6)	175 (0.7)		<20	14 (0.1)	
Other	9 (0.6)	114 (0.5)		135 (8.2)	818 (8.6)	
Diabetes duration, mean ± SD, years	13.4 ± 9.4	7.5 ± 8.2	4.95×10^{-141}	11.0 ± 6.3	7.5 ± 4.8	9.36×10^{-146}
Current smoking, n (%)	132 (9.0)	2,840 (11.6)	2.2×10^{-3}	4 (0.2)	101 (1.1)	2.50×10^{-3}
Systolic blood pressure, mean ± SD, mmHg	148.7 ± 18.7	142.3 ± 18.1	1.04×10^{-3}	125.4 ± 11.7	127.8 ± 10.9	0.38
Diastolic blood pressure, mean ± SD, mmHg	79.2 ± 11.3	82.7 ± 10.5	2.53×10^{-3}	74.7 ± 6.3	76.5 ± 6.1	0.25
BMI, mean ± SD, kg/m^2	31.9 ± 5.8	31.6 ± 5.8	0.08	33.0 ± 15.8	33.7 ± 10.2	0.02
BMI categories, n (%), kg/m^2						
<25	129 (8.8)	2,281 (9.3)	0.10	216 (13.2)	1,032 (10.8)	4.86×10^{-5}
25–29.9	478 (32.5)	8,497 (34.8)		473 (28.9)	2,386 (25)	
≥30.0	864 (58.7)	13,646 (55.9)		935 (57.0)	5,887 (61.8)	
Coronary artery disease, n (%)	614 (41.7)	5,200 (21.3)	3.40×10^{-74}	525 (32.0)	1,906 (20.0)	1.75×10^{-27}
Chronic kidney disease, n (%)	386 (26.2)	1,501 (6.1)	1.03×10^{-181}	623 (38)	1,536 (16.1)	4.30×10^{-95}
Hypertension, n (%)	1,259 (85.6)	13,316 (54.5)	4.18×10^{-120}	1479 (90.2)	7,646 (80.3)	6.80×10^{-22}
HbA_{1c}, mean ± SD, %	7.7 ± 1.6	7.0 ± 1.2	1.12×10^{-98}	8.0 ± 1.7	7.0 ± 1.6	3.95×10^{-86}
Glucose, mean ± SD, mg/dL	163.2 ± 80.8	133.8 ± 58.0	1.31×10^{-64}	159 ± 65.5	117.4 ± 47.5	1.85×10^{-17}
Triglycerides, mean ± SD, mg/dL	89.4 ± 112.2	199.5 ± 114.7	1.46×10^{-3}	165 ± 127.8	160 ± 118.3	0.21
LDL, mean ± SD, mg/dL	98.9 ± 29.2	110.1 ± 33.3	7.75×10^{-34}	88.4 ± 35.9	97.9 ± 34	5.37×10^{-9}
Creatinine, mean ± SD, mg/dL	1.0 ± 0.8	0.8 ± 0.3	4.27×10^{-93}	1.4 ± 1.7	1.1 ± 1.4	9.49×10^{-20}

[a]Individuals with retinopathy of all ancestries were compared with those without retinopathy across demographic factors, clinical variables, and hospital laboratory tests using a two-sided t test for continuous variables and χ^2 test for categorical variables.

noncarriers (females: CT/TT 6.3% vs. CC: 6.5%; males: T: 13.6% vs. C: 8.8%; differences were not statistically significant; Supplementary Fig. 2). When stratified by ngA1cPS, retinopathy prevalence was higher for individuals below the 50th percentile compared with above the 50th percentile of the ngA1cPS, regardless of sex or *G6PD* genotype. Among individuals with HbA_{1c} greater than 7%, there were no clear differences in retinopathy prevalence across the ngA1cPS or *G6PD* genotype (Fig. 3).

CONCLUSIONS

This study provided a large, population-scale, multiancestry examination of polygenic nonglycemic HbA_{1c} effects on retinopathy risk among individuals with diabetes. We showed that individuals with diabetes who were genetically predisposed to having lower measured HbA_{1c} due to nonglycemic genetic effects had a higher prevalence of retinopathy. The higher prevalence of retinopathy in the bottom quintile of the ngA1cPS, compared with above the quintile, was observed despite similar measured HbA_{1c} across the ngA1cPS. The OR of the ngA1cPS bottom quintile did not attenuate when adjusting for known retinopathy risk factors, including measured HbA_{1c}, diabetes duration, hypertension, random glucose, triglycerides, LDL, creatinine, and smoking.

We found an equivalent increased risk of retinopathy among individuals in the bottom quintile of the ngA1cPS for both African American/African British and European ancestries. These findings were replicated in two populations that differed by continent, ancestral diversity, baseline comorbidities, and distribution of retinopathy risk factors. This suggests that precision diabetes care should be tailored based on an individual's unique genetic profile and not only on discrete ancestry or geography. We emphasize that it would be inappropriate, and potentially harmful, to apply race-based or ancestry-based HbA_{1c} diagnostic thresholds or targets to an entire race or genetically inferred ancestral population without accounting for the full complement of genetic effects on nonglycemic variation of HbA_{1c}, as evidenced by the clinically meaningful differences in retinopathy prevalence across the ngA1cPS within ancestries.

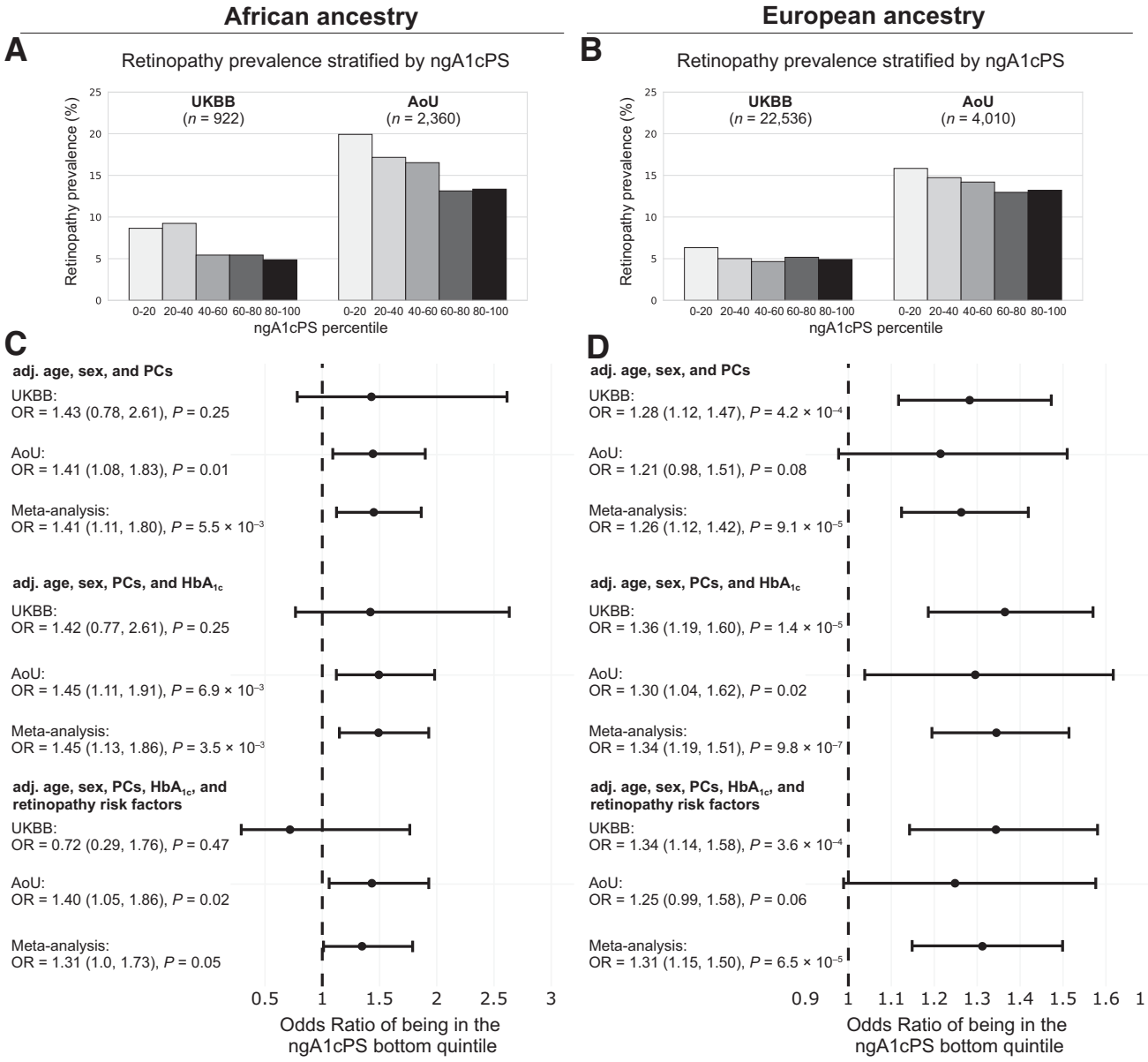

Figure 1—Association of ngA1cPS with retinopathy among diabetes cases in African American/African British and European ancestries in UKBB and AoU. *A*: Retinopathy prevalence stratified by quintiles of the ngA1cPS in African American/African British ancestry samples. *B*: European ancestry samples. *C*: Forest plots of ORs in UKBB and AoU, and a meta-analysis of the two effect estimates for the bottom 20% compared with the middle 60% of the ngA1cPS in African American/African British ancestry samples, adjusted for age, sex, and principal components (PCs), then additionally adjusted for measured HbA_{1c}, and finally adjusted for other retinopathy risk factors including diabetes duration, chronic kidney disease, hypertension, glucose, triglycerides, LDL, creatinine, and current smoking status. *D*: European ancestry samples. Effect estimates are reported as ORs with 95% CIs and *P* values. adj., adjusted; African ancestry, African American/African British ancestry.

Our results suggest that the clinical management of patients with diabetes could be improved by considering genetic effects on HbA_{1c}. These results differ from other disease prediction analyses involving genetic and clinical variables, whereby the marginal value of the genetic information declines as we consider relevant clinical measurements (26,27). We infer that the risk of other diabetes-related outcomes that depend on glycemic control could be better estimated if HbA_{1c} genetics are considered, and if ignored, could result in disparities in outcomes. Genetically determined nonglycemic variation in HbA_{1c} may partly explain why some individuals who achieve HbA_{1c} glycemic targets still develop complications. Although this study was focused on individuals with diagnosed type 2 diabetes, our findings have implications on the use of HbA_{1c} in the management of other types of diabetes, including type 1 diabetes. These results also raise the questions of whether the broad application of HbA_{1c} testing in diverse populations to diagnose diabetes without confirmation with glucose measurements is appropriate, and of the use of simple linear regression equations for translating HbA_{1c} measurements to average glucose (28,29) without considering genetic effects that may alter the HbA_{1c}-glycemia relationship.

Fructosamine and other glycated proteins (30,31) can be used in clinical practice in the presence of genetic or

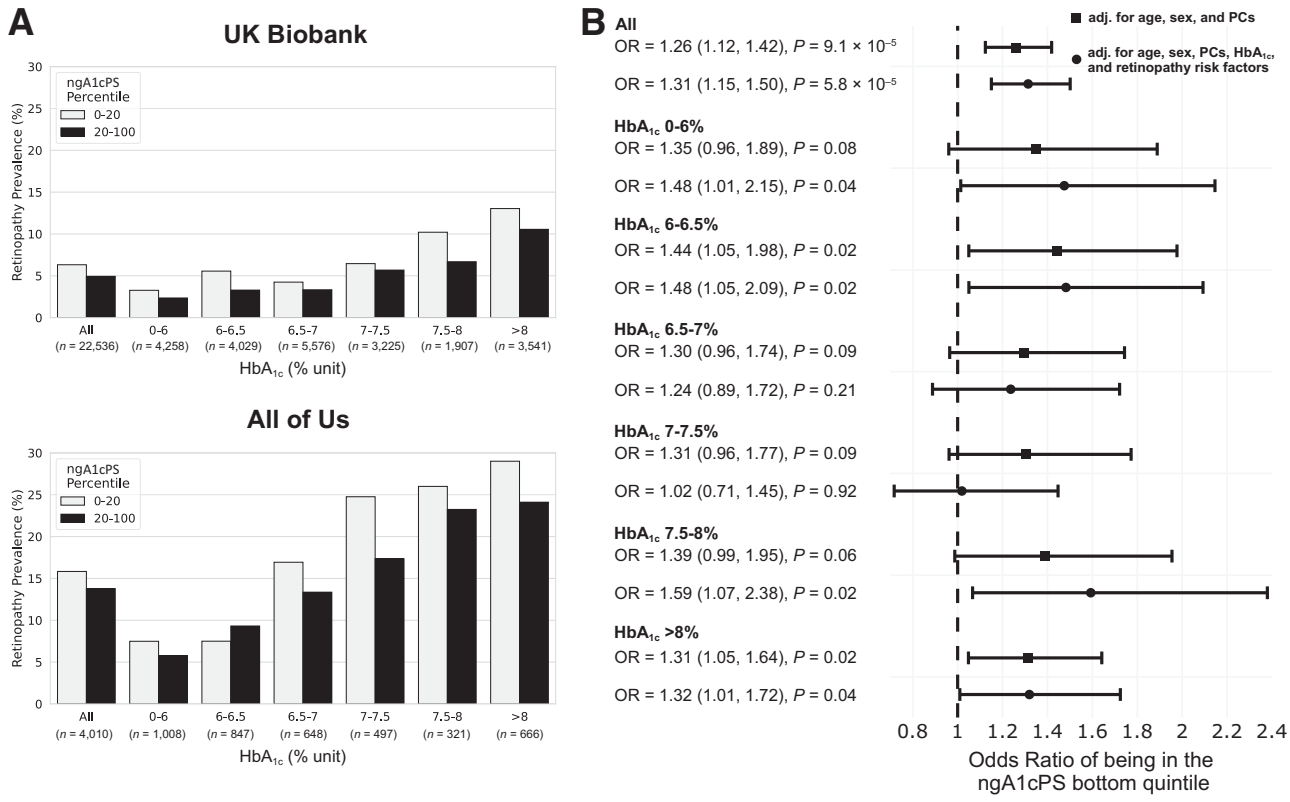

Figure 2—Association of the ngA1cPS with retinopathy among diabetes cases of European ancestry in UKBB and AoU stratified by HbA$_{1c}$ (%). *A*: Retinopathy prevalence stratified by the bottom 20% and top 80% of the ngA1cPS and by measured HbA$_{1c}$ categories (<6%, 6–6.5%, 6.5–7%, 7–7.5%, 7.5–8%, and >8%) in African American/African British ancestry samples. *B*: Forest plots of UKBB and AoU meta-analytic ORs for the bottom 20% compared with the middle 60% of the ngA1cPS in African American/African British ancestry samples by HbA$_{1c}$ categories, adjusted for age, sex, and principal components, then additionally adjusted for diabetes duration, chronic kidney disease, hypertension, glucose, triglycerides, LDL, creatinine, and current smoking status. Effect estimates are reported as ORs with 95% CIs and *P* values. adj., adjusted; PC, ancestry principal component.

nongenetic factors that meaningfully interfere with HbA$_{1c}$ measurement (e.g., hemoglobin variants and renal failure) or interpretation (e.g., pregnancy, anemia, recent blood loss). Yet, these glycated proteins have other limitations: their measurements are affected by albumin production, reflect glycemia over a short time, and have not been as extensively evaluated for their prediction of long-term complications of diabetes as has measured HbA$_{1c}$ (32,33). Time in range from continuous glucose monitoring (CGM) is also highly correlated with HbA$_{1c}$ and can be used as an outcome measure or predictor of diabetes-related complications (34–37). However, as the clinical use of CGM is often restricted to only patients requiring frequent glucose monitoring; CGM is unlikely to replace HbA$_{1c}$ completely in diabetes screening or prevention of complications in general populations.

In the stratified analysis by measured HbA$_{1c}$ categories in European ancestry, the excess risk conferred by the ngA1cPS was mostly driven by the individuals with

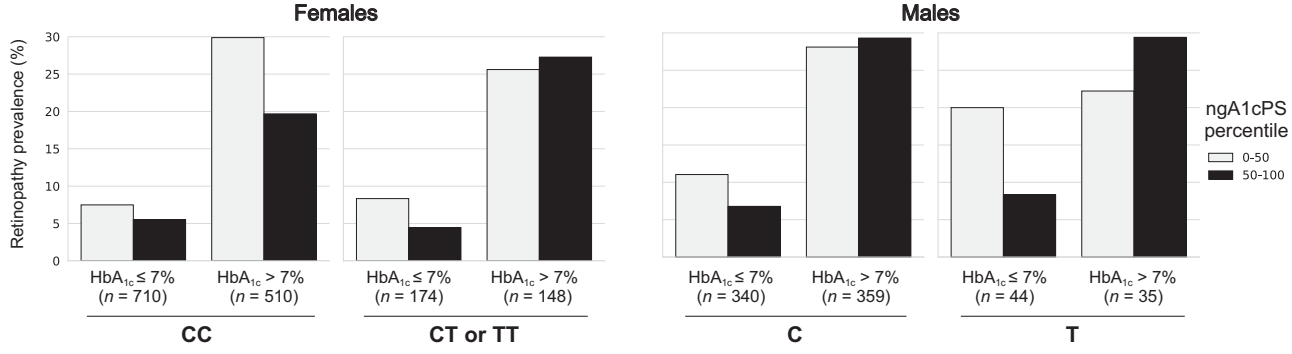

Figure 3—Retinopathy prevalence stratified by genetic sex, *G6PD* genotype, HbA$_{1c}$, and ngA1cPS among diabetes cases of African American ancestry in AoU. Retinopathy prevalence is stratified by genetic sex (female vs. male), *G6PD* genotype (CC vs. CT or TT), HbA$_{1c}$ (below vs. >7%), and ngA1cPS (<50th percentile vs. >50th percentile).

HbA_{1c} between 6% and 6.5%, around the diagnostic threshold for diabetes, and between 7.5% and 8%, reflecting suboptimal glycemic control. HbA_{1c} genetics may have less of an impact on retinopathy risk in the nondiabetic range where its prevalence is low. In individuals with HbA_{1c} above 8%, lenient glycemic targets in the presence of comorbid conditions, psychosocial barriers to diabetes management, or use of home glucose monitoring may be more important determinants of complications risk than HbA_{1c} genetics.

Expectedly, the proportion of individuals on metformin or insulin use was three to four times higher among those with HbA_{1c} greater than 7% compared with those with HbA_{1c} less than 6%. Yet, the use of these medications was similar across the ngA1cPS regardless of HbA_{1c} category—a reflection of current clinical practice that does not consider genetic effects when using measured HbA_{1c} to guide treatment decisions. Individuals in the middle quintiles and top quintile of the ngA1cPS with HbA_{1c} between 6.5% and 7% had similar retinopathy prevalence as those in the bottom quintile with HbA_{1c} between 6% and 6.5%. If differences in retinopathy prevalence were due to undertreatment, individuals in the bottom quintile will need to have HbA_{1c} below 6.5% to have a similar risk for retinopathy as their counterparts above this quintile with HbA_{1c} between 6.5% and 7%.

Among individuals of African ancestry with HbA_{1c} below 7%, retinopathy prevalence was higher in *G6PD* carriers compared with noncarriers, though differences were not statistically significant. The large HbA_{1c}-lowering effect of the *G6PD* variant likely resulted in underdiagnosis of diabetes and its complications among carriers, reducing the power in our analysis. Indeed, the diabetes prevalence in *G6PD* carriers versus noncarriers was 8% versus 11% in males (P = 0.006) and 14% versus 16% in females (P = 0.02). Nevertheless, regardless of sex or *G6PD* carrier status, retinopathy prevalence was higher below the 50th percentile compared with those above the 50th percentile of the ngA1cPS. We concluded that the ngA1cPS, uniquely constructed for each ancestry, captured polygenic effects that represented a more comprehensive estimation of the genetic risk in people with diabetes. Still, we acknowledge that these ngA1cPS only included genetic variants reported in published GWAS and do not fully account for all genetic effects across the genome on nonglycemic variation in HbA_{1c}, such as rare or low-frequency variants.

Apart from undertreatment of hyperglycemia and delayed diabetes diagnosis, other factors could account for these differences in retinopathy prevalence across the ngA1cPS. The ngA1cPS could be associated with unmeasured variables or retinopathy risk factors that were unaccounted for in our analysis. Because genetic effects were modeled as polygenic scores, we were unable to distinguish among the various nonglycemic mechanisms, including a propensity for glycation, sometimes referred to the hemoglobin glycation index, which is associated with retinopathy and other outcome measures (38–40). Notably, a significant proportion of the contributing genetic variants affected erythrocytic or reticulocyte parameters (Supplementary Table 3), which suggests that the principal mechanism within ancestry was likely differences in erythrocytic turnover. By including genetic variants that had up to 25% probability of being assigned "glycemic" in the soft clustering analysis (15), the ngA1cPS may have included variants that were modestly associated with glycemic traits. Nonetheless, if the ngA1cPS included variants associated with glycemia, the observed association between lower ngA1cPS and higher risk of retinopathy would have been biased toward the null, because variants that raise HbA_{1c} through hyperglycemia are expected to increase, and not reduce, retinopathy risk. Future studies that involve multiple glucose measurements, such as continuous glucose monitoring, and treatment exposures over time could help clarify the mechanisms giving rise to these differences in retinopathy prevalence.

Similar to previous analyses of diabetic retinopathy, this study was limited by the precision with which diabetes and retinopathy could be defined by ICD coding. We recognize that the ngA1cPS was composed of variants reported to be associated with HbA_{1c} in a multiancestry GWAS meta-analysis of diabetes-free individuals, which could explain why genetic effects were attenuated in people with HbA_{1c} higher than 6.5%. Nevertheless, it is reasonable to assume that genetic effects on nonglycemic variation in HbA_{1c} do not vary by underlying glycemia or diabetes status. Although an analysis of incident retinopathy would be useful to evaluate the added value of ngA1cPS in the prediction of future retinopathy for people with newly diagnosed diabetes, we recognize the potential for detection bias in a time-to-event analysis. Compared with people with a lower ngA1cPS, those with a higher ngA1cPS are expected to have higher measured HbA_{1c} for the same average glucose and, therefore, are more likely to be diagnosed with diabetes, screened for diabetes-related complications, and be diagnosed with retinopathy, whereas people with a lower ngA1cPS are more likely to have undetected hyperglycemia and diabetes-related complications.

In a sensitivity analysis, we tested the association of a ngA1cPS composed of all 122 genetic variants with measured HbA_{1c} and retinopathy in African American ancestry, and effect estimates obtained were consistent with the ngA1cPS composed of only 14 genetic variants. Per SD of the ngA1cPS, the estimated odds of retinopathy were lower by 14% in African ancestry and by 9% in European ancestry (Supplementary Table 7). In people without the *G6PD* variant, the mean of the ngA1cPS composed of all 122 genetic variants was 0.26% higher in African ancestry compared with European ancestry, supporting the hypothesis that ancestral differences in mean HbA_{1c} may be explained, to some extent, by genetics (Supplementary Table 5). Given the poor transferability of the large number of genetic variants, and the different number of variants contributing to the ngA1cPS for each ancestry, we refrain from making any firm conclusions from ancestral differences. The lack of transferability could be explained by differences in linkage disequilibrium resulting in some of the causal variants tagged by lead variants in European ancestry but not tagged by the same variants in African ancestry. Furthermore, the discovery of nonglycemic HbA_{1c} variants in multiancestry GWAS meta-analysis is biased toward European ancestry because the contribution of African ancestry samples was much smaller. We also recognize that genetically inferring African American/African British ancestry does not capture the full genetic diversity of the Africa continent. We would expect that, as larger GWAS of HbA_{1c} are conducted in diverse populations, more HbA_{1c} variants will be discovered, enabling the construction of more comprehensive ngA1cPS in non-European ancestries and a fairer

comparison of the impact of genetics on complication risks between ancestries.

In sum, our study showed that the aggregate effect of variants that lower HbA_{1c} independently of glycemia is associated with higher odds of retinopathy in individuals of African American/African British ancestry as well as European ancestry. Genetic effects need to be considered to define personalized HbA_{1c} targets, reduce disparities in diabetes-related outcomes, and promote equal care for people of all genetic backgrounds.

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