Common variants at 10 genomic loci influence hemoglobin A(C) levels via glycemic and nonglycemic pathways

Common Variants at 10 Genomic Loci Influence Hemoglobin A_{1C} Levels via Glycemic and Nonglycemic Pathways

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OBJECTIVE—Glycated hemoglobin (HbA_{1c}), used to monitor and diagnose diabetes, is influenced by average glycemia over a 2- to 3-month period. Genetic factors affecting expression, turnover, and abnormal glycation of hemoglobin could also be associated with increased levels of HbA_{1c}. We aimed to identify such genetic factors and investigate the extent to which they influence diabetes classification based on HbA_{1c} levels.

RESEARCH DESIGN AND METHODS—We studied associations with HbA_{1c} in up to 46,368 nondiabetic adults of European descent from 23 genome-wide association studies (GWAS) and 8 cohorts with de novo genotyped single nucleotide polymorphisms (SNPs). We combined studies using inverse-variance meta-analysis and tested mediation by glycemia using conditional analyses. We estimated the global effect of HbA_{1c} loci using a multilocus risk score, and used net reclassification to estimate genetic effects on diabetes screening.

RESULTS—Ten loci reached genome-wide significant association with HbA_{1c}, including six new loci near *FN3K* (lead SNP/*P* value, rs1046896/$P = 1.6 \times 10^{-26}$), *HFE* (rs1800562/$P = 2.6 \times 10^{-20}$), *TMPRSS6* (rs855791/$P = 2.7 \times 10^{-14}$), *ANK1* (rs4737009/$P = 6.1 \times 10^{-12}$), *SPTA1* (rs2779116/$P = 2.8 \times 10^{-9}$) and *ATP11A/TUBGCP3* (rs7998202/$P = 5.2 \times 10^{-9}$), and four known HbA_{1c} loci: *HK1* (rs16926246/$P = 3.1 \times 10^{-54}$), *MTNR1B* (rs1387153/$P = 4.0 \times 10^{-11}$), *GCK* (rs1799884/$P = 1.5 \times 10^{-20}$) and *G6PC2/ABCB11* (rs552976/$P = 8.2 \times 10^{-18}$). We show that associations with HbA_{1c} are partly a function of hyperglycemia associated with 3 of the 10 loci (*GCK*, *G6PC2* and *MTNR1B*). The seven nonglycemic loci accounted for a 0.19 (% HbA_{1c}) difference between the extreme 10% tails of the risk score, and would reclassify ~2% of a general white population screened for diabetes with HbA_{1c}.

CONCLUSIONS—GWAS identified 10 genetic loci reproducibly associated with HbA_{1c}. Six are novel and seven map to loci where rarer variants cause hereditary anemias and iron storage disorders. Common variants at these loci likely influence HbA_{1c} levels via erythrocyte biology, and confer a small but detectable reclassification of diabetes diagnosis by HbA_{1c}. ***Diabetes*** **59: 3229–3239, 2010**

Glycated hemoglobin (HbA_{1c}) results from glycation, the nonenzymatic and mostly irreversible chemical modification by glucose of hemoglobin molecules carried in erythrocytes. The rate of glycation directly depends on ambient blood glucose levels, so HbA_{1c} reflects the average concentration of blood glucose over the average life span of a erythrocyte (in humans, ~3 months), and represents a longer-term indicator of glycemic status compared to fasting glucose (FG) (1). In addition to ambient glycemia, it is known that medical conditions that change erythrocyte turnover (such as hemolytic anemias, chronic malaria, major blood loss, or blood transfusion), as well as genetic hereditary anemias and iron storage disorders (caused by rare variants in genes involved in erythrocyte membrane stability, hemoglobin function, erythrocyte glucose sensing, and membrane transport) may influence the variability of HbA_{1c} in populations (2–4).

Common genetic variation also influences HbA_{1c} variability. The heritability of HbA_{1c} levels is relatively high (47–59%) when compared with FG (34–36%) or glucose levels as determined by 2-h postoral glucose tolerance test (33%) (5,6). Recent genome-wide association studies (GWAS) of FG have shown that single nucleotide polymorphisms (SNPs) near three loci (*G6PC2*, *MTNR1B*, and *GCK*) are also associated with HbA_{1c} levels (7–15). A GWAS for HbA_{1c} levels in 14,618 nondiabetic women found a suggestive association ($P = 9.8 \times 10^{-8}$) with *SLC30A8* (a known type 2 diabetes locus) and genome-wide significant association ($P < 5 \times 10^{-8}$) at a novel locus, *HK1*, where rare variants are known to be associated with nonspherocytic hemolytic anemia (16). This suggests that both glycemic and erythrocyte genetic factors are associated with variation in HbA_{1c}, but a more thorough accounting of common variants comprising the genetic architecture of HbA_{1c} is needed.

In this study we tested the hypothesis that additional common genetic factors are associated with HbA_{1c}. We conducted a meta-analysis of GWAS in up to 46,368 nondiabetic individuals of European ancestry as part of the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) effort. In addition to seeking new common variants affecting HbA_{1c} levels, we sought to place the size of the effect of novel genetic findings into the

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TABLE 1
Characteristics of 46,368 participants from 31 cohorts employed in the meta-analysis

Cohort	*N* males/females	Age (years) men/women	BMI (kg/m^2) men/women	HbA_{1C} (%, NGSP) men/women	Fasting plasma glucose (mmol/l) men/women
ARIC	3,106/3,671	57.4 (5.7)/56.7 (5.6)	27.33 (3.89)/26.63 (5.30)	5.41 (0.38)/5.37 (0.36)	5.75 (0.50)/5.52 (0.50)
B58C-T1DGC	1,217/1,284	45.3 (0.3)/45.2 (0.3)	27.93 (4.12)/26.86 (5.5)	5.18 (0.48)/5.22 (0.51)	—
B58C-WTCCC	711/717	44.9 (0.4)/44.9 (0.4)	27.79 (4.21)/26.84 (5.41)	5.21 (0.68)/5.21 (0.51)	—
BLSA	253/235	72.2 (13.5)/67.2 (15.6)	26.99 (3.92)/25.87 (4.94)	5.44 (0.53)/5.45 (0.45)	5.25 (0.56)/4.99 (0.48)
Croatia	275/384	54.8 (15.0)/55.2 (15.8)	27.43 (3.65)/26.94 (4.59)	5.25 (0.49)/5.31 (0.55)	5.40 (0.66)/5.26 (0.65)
deCODE	170/172	66.1 (14.4)/63.8 (16.0)	28.20 (4.00)/28.0 (4.90)	5.80 (0.95)/5.77 (1.25)	—
DESIR	178/538	53.1 (5.6)/49.5 (8.5)	23.15 (1.16)/21.36 (1.85)	5.25 (0.38)/5.16 (0.38)	5.11 (0.32)/5.01 (0.38)
DGI	218/262	59.1 (10.6)/59.5 (10.6)	26.42 (3.12)/26.29 (4.29)	5.73 (0.56)/5.61 (0.59)	5.50 (0.52)/5.39 (0.45)
DIAGEN	429/571	59.0 (14.2)/59.0 (15.4)	27.08 (3.61)/26.80 (4.82)	5.59 (0.65)/5.50 (0.61)	—
Epic 5,000	1,732/1,627	57.6 (9.4)/54.0 (9.0)	25.75 (2.60)/24.84 (3.38)	5.19 (0.55)/5.08 (0.55)	—
EPIC cases	409/548	60.8 (8.9)/60.2 (9.1)	32.55 (2.53)/33.44 (3.24)	5.58 (0.97)/5.47 (0.62)	—
EPIC cohort	859/1,052	61.3 (9.3)/60.0 (9.2)	26.79 (3.31)/26.33 (4.36)	5.38 (0.56)/5.32 (0.57)	—
Fenland	606/772	44.4 (7.4)/45.4 (7.2)	27.56 (3.91)/26.59 (5.35)	5.42 (0.37)/5.37 (0.37)	5.01 (0.47)/4.74 (0.48)
FHS	886/1,110	54.7 (10.0)/54.1 (9.9)	27.85 (3.92)/26.13 (4.97)	5.24 (0.62)/5.25 (0.61)	5.36 (0.48)/5.14 (0.49
GenomeEUtwin	0/568	—/55.1 (21.8)	—/24.6 (4.18)	—/5.11 (0.68)	—/5.24 (0.55)
HEALTH2000	580/625	49.1 (10.4)/51.7 (11.4)	25.69 (3.26)/25.32 (4.19)	5.22 (0.29)/5.06 (0.32)	5.34 (0.48)/5.17 (0.42)
Lolipop	582/188	53.2 (10.4)/51.2 (10.5)	27.49 (3.92)/26.74 (5.33)	5.05 (0.54)/5.34 (0.38)	5.51 (1.45)/5.35 (1.75)
LURIC	215/195	54.1 (12.6)/61.1 (11.1)	26.90 (3.60)/26.60 (4.00)	5.80 (0.60)/5.90 (0.60)	—
KORA F3	711/751	62.3 (10.2)/61.6 (10.1)	27.89 (3.49)/27.70 (4.95)	5.30 (0.38)/5.33 (0.31)	—
KORA S4	844/892	53.9 (8.9)/53.4 (8.8)	27.91 (3.87)/27.25 (4.89)	5.57 (0.46)/5.59 (0.45)	—
METSIM	1,789/0	57.0 (7.3)/—	26.63 (3.76)/—	5.56 (0.32)/—	—
NHANES III	468/746	51.3 (20.6)/51.4 (20.3)	26.92 (4.85)/26.37 (5.81)	5.38 (0.50)/5.15 (0.55)	—
NTR	513/939	47.7 (14.4)/43.3 (13.7)	25.58 (3.28)/24.59 (4.02)	5.27 (0.46)/5.28 (0.45)	5.53 (0.51)/5.32 (0.51)
ORCADES	298/353	53.7 (15.3)/52.2 (15.4)	27.79 (4.14)/27.30 (5.11)	5.40 (0.49)/5.41 (0.51)	5.45 (0.51)/5.18 (0.49)
Partners/Roche	291/357	52.7 (12.9)/52.5 (12.7)	27.80 (5.20)/27.10 (7.30)	5.49 (0.48)/5.47 (0.45)	—
PROCARDIS	687/144	60.5 (6.7)/62.8 (6.3)	27.65 (3.58)/28.11 (4.98)	5.98 (1.07)/6.17 (1.10)	—
SardiNIA	1,418/1,928	46.5 (17.1)/45.2 (16.0)	26.36 (3.99)/24.65 (4.82)	5.47 (0.52)/5.39 (0.45)	4.96 (0.59)/4.67 (0.54)
SHIP	1,696/1,842	49.0 (16.0)/47.0 (16.0)	27.30 (3.90)/26.60 (5.20)	5.3 (0.60)/5.2 (0.60)	—
Sorbs	254/376	46.6 (16.2)/46.4 (15.8)	26.90 (3.60)/26.7 (5.50)	5.35 (0.37)/5.36 (0.38)	5.47 (0.49)/5.21 (0.57)
SardiNIA stage2	555/890	46.4 (15.1)/46.3 (15.8)	26.36 (3.48)/24.61 (4.60)	5.45 (0.85)/5.31 (0.86)	—
Segovia	274/309	53 (12)/55 (12)	27.35 (3.15)/27.41 (4.68)	5.17(0.49)/5.17 (0.45)	—

Data are mean (SD). Fifteen cohorts were included in the fasting-glucose adjusted analysis shown in Table 2 (ARIC, BLSA, CROATIA, Fenland, FHS, DESIR, GENOMEUTWIN, Lolipop, NTR, ORCADES, SardiNIA, KORA F4, DGI, Sorbs and Health2000). BLSA, DGI, Fenland, FHS, KORA F4 and Sorbs were used for analyses that included 2-h glucose. The mean (mmol/l), SE and *N* for 2-h glucose levels for males and females, respectively, were: 6.96 (2.47) (236)/6.42 (2.04) (207) in BLSA; 5.75 (1.20) (209)/6.15 (1.25) (254) in DGI; 5.27 (1.41) (600)/5.16 (1.35) (757) in Fenland, 5.744 (1.614) (858)/5.992 (1.707) (1,067) in FHS, and 5.19 (2.02) (254)/5.54 (1.96) (376) in Sorbs. Fasting glucose was not available in KORA S4, thus conditional models were run in KORA F4, a follow-up visit of KORA S4 samples. Mean and SE 2-h glucose levels in males and females, respectively, were: 5.66 (0.67)/5.60 (0.57) for HbA_{1C} and 5.82 (1.20)/5.40 (1.01) for glucos**e.** Cohorts in italics provided only de novo genotyping data). The means for Hb (g/l, males/females) were 148.39 (10.29)/135.94 (9.55) (KORA F3), 148.21 (10.00)/134.51 (9.15) (KORA F4), 152.38 (11.33)/136.56 (10.38) (NHANES III) and 148.54 (12.12)/130.83 (11.60) (SardiNIA). The means for MCV (pg, males/females) were 92.32 (3.91)/90.74 (4.08) (KORA F3), 92.04 (4.23)/90.83 (4.38) (KORA F4), 89.69 (4.45)/89.40 (4.34) (NHANES III) and 87.29 (9.28)/85.64 (9.22) (SardiNIA). The means for MCH (fl, males/females) were 31.22 (1.51)/30.60 (1.64) (KORA F3), 31.50 (1.62)/30.89 (1.73) (KORA F4), 30.50 (1.74)/30.22 (1.67) (NHANES III) and 29.14 (3.60)/28.40 (3.69) (SardiNIA). The means for Iron (imol/l, males/females) were 17.66 (5.34)/16.29 (5.25) (KORA F3), 22.41 (6.87)/20.53 (6.53) (KORA F4), 18.78 (6.53)/17.03 (6.96) (NHANES III) and 18.01 (6.23)/15.30 (5.98) (SardiNIA). The means for Transferrin (g/l, males/females) were 2.45 (0.33)/2.56 (0.36) (KORA F3), 2.51 (0.35)/2.54 (0.38) (KORA F4), n.a. (NHANES III) and 1.96 (0.52)/2.07 (0.579) (SardiNIA).

population perspective of diabetes screening and diagnosis. HbA_{1c} levels have recently been recommended for this use based on high overlap between HbA_{1c} distributions in populations without diabetes and those with subclinical (undiagnosed) diabetes, ease of measurement, and an established role as a treatment target in clinical diabetes (17,18). We estimated the degree to which these HbA_{1c}-associated loci shifted the population level distribution of HbA_{1c}, and thereby influenced diabetes screening using HbA_{1c}.

RESEARCH DESIGN AND METHODS

Cohort description, study design, and genotyping. The cohorts included in this study were part of MAGIC (19). The characteristics of the population samples used in this analysis are shown in Table 1. All participants were adults of European ancestry from Europe or the U.S., and free of diabetes as assessed by either clinical diagnosis, self-reported diabetes, diabetes treatment, or undiagnosed diabetes defined by FG ≥7.0 mmol/l. HbA_{1c} (in percentages) was measured in all studies from fasting or nonfasting whole blood using NGSP-certified methods. We found remarkably consistent means and SD across studies, increasing confidence that laboratory variability had a minimal effect on the study results. A local research ethics committees approved all studies and all participants gave informed consent.

We carried out a meta-analysis including 35,920 participants from 23 cohorts with available HbA_{1c} measurements and genotype data including ~2.5M genotyped and imputed autosomal SNPs. This sample size ensures 80% power to detect SNPs, explaining 0.12% of the trait variance at $\alpha = 5 \times 10^{-8}$. For 5 SNPs (rs1046896, rs16926246, rs1799884, rs1800562, and rs552976) that had been previously selected from an interim analysis of the first 10 participating cohorts (n = 14,898), we obtained further data by genotyping up to 10,448 participants from 8 additional cohorts. The sample size for each SNP is thus related to the number of cohorts that were genotyped (up to 31) and to the specific call rate. Details on genotyping methodology, quality control metrics, and statistical analyses for each

cohort are shown in supplementary Table S1 in the online appendix available at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0502/DC1. Additional details on imputation and quality control applied by each study are given in the online supplementary METHODS.

Primary genome-wide association studies and meta-analysis. In each cohort a linear regression model was fit using untransformed (percentage) HbA_{1c} as the dependent variable to evaluate the additive effect of genotyped and imputed SNPs. HbA_{1c} showed a mild deviation from normality in the majority of cohorts. Log-transformation did not significantly improve normality; nevertheless, such mild deviation did not result in an inflation of the test statistics suggestive of an excess of false positives, as indicated by a genomic correction λ very close to the expected value of 1.0; thus, we report untransformed (percentage) HbA_{1c} results. The model was adjusted for age, sex, and other cohort-specific variables as applicable. Further details are given in the supplementary METHODS and supplementary Table S1. Regression estimates for each SNP were combined across studies in a meta-analysis using a fixed effect inverse-variance approach (justified by nonsignificant heterogeneity of effect sizes at all validated loci), as implemented in the METAL software. The individual cohort analysis results were corrected prior to performing the meta-analysis for residual inflation of the test statistic using the genomic control method if the λ coefficient was >1.0 (20). Cohort-specific results for each of the 10 loci are given in supplementary Table S2. Heterogeneity across study-specific effect sizes was assessed using the standard χ^2 test implemented in METAL, Cochran's Q statistic and the I^2 statistics (21).

Association with related traits and diseases. Secondary analyses were carried out on 10 SNPs (rs2779116, rs552976, rs1800562, rs1799884, rs4737009, rs16926246, rs1387153, rs7998202, rs1046896, and rs855791) reaching genome-wide significance and including only the stronger of the 2 significant *ANK1* SNPs (see supplementary METHODS for additional information). A first goal was to detect "pleiotropic" effects on potentially related traits for the 10 loci. To this end we tested them for association with correlated intermediate traits (BMI, and glycemic and hematologic parameters, supplementary Table S3).

Further, we carried out association analyses of HbA_{1c} levels conditional on FG levels (Table 3) and hematologic parameters (supplementary Table S4) to formally test mediation by glycemia or erythrocyte traits. Mediation is used here to distinguish it from confounding. A confounder is a characteristic associated with both exposure and outcome but is not on the causal pathway linking the two together. By contrast, a mediator is also associated with both exposure and outcome, but is on the causal pathway that may explain the association between them. Our mediation analyses decompose the association between a SNP and HbA_{1c} into two paths. The first path links the SNP directly to HbA_{1c}, and the second path links the SNP to HbA_{1c} through a mediator, e.g., FG or hematologic parameters. A marked attenuation of the size of effect on HbA_{1c} of the SNP in the conditional "mediation" model implies that the SNP (e.g., rs552976) acts on the mediator (e.g., FG), which in turn acts on HbA_{1c} levels. Further details on these analyses are provided in the on-line supplementary METHODS.

Finally, we tested associations of the 10 loci with risk of type 2 diabetes or coronary artery disease (CAD) using adequately powered case-controlled meta-analyses. Association statistics with type 2 diabetes were obtained from a previous analysis of the MAGIC datasets or from the DIAGRAM+ meta-analysis (22). CAD associations were tested in this study using cohorts described in supplementary Table S5. The CAD analytic sample size assembled for this study had 80% power to detect associations at an α level of 5×10^{-8} for a genotype relative risk of 1.14, and a risk allele frequency of 0.2.

Estimates of genetic effect size. We used several methods to evaluate the size of the genetic effect of HbA_{1c}-associated SNPs: *1*) we used regression to estimate in percentages the total variance in HbA_{1c} explained by the 10 loci; *2*) we calculated an additive genotype score based on the number of risk alleles at 7 (nonglycemic) or 10 (all) loci and then calculated the difference in HbA_{1c} (%) between individuals in the top 10% of the genotype score distribution and those in the bottom 10% (supplementary METHODS); and *3*) we used net reclassification analysis to gauge the effect of individual genotype on HbA_{1c} distributions at the population level.

Net reclassification analysis. Variation in the measured level of HbA_{1c} associated with nonglycemic genetic effects may affect the classification of individuals as diabetic or nondiabetic when screening general population samples using HbA_{1c}. We used this relationship as a way to understand the clinical influence of the HbA_{1c} loci when applied at the population level. We estimated the change in classification that occurred when accounting for effects of the seven loci presumed not to affect HbA_{1c} via primarily glycemic mechanisms (*SPTA1*, *HFE*, *ANK1*, *HK1*, *ATP11A/TUBGCP3*, *FN3K*, and *TMPRSS6*) using the method of Pencina et al. (23). For this analysis we combined the Framingham Heart Study (FHS), and Atherosclerosis Risk In Communities (ARIC) European ancestry cohorts (N = 10,110). ARIC and FHS have several characteristics suitable for this analysis: *1*) they are population-based samples, thus allowing a test of diabetes screening in a truly unselected sample; *2*) they are of large sample size, thus maximizing the number of diabetic subjects that can readily be folded back for reclassification analysis; *3*) they have both fasting glucose and HbA_{1c} measured. We excluded as in previous analyses all individuals on diabetes treatment (diagnosed diabetes), but retained individuals with FG ≥7.0 mmol/l not on treatment (who we classified as having undiagnosed diabetes, N = 593) as well as all nondiabetic individuals (N = 9,517). We then sought to differentiate these individuals on the basis of their HbA_{1c} levels, using ≥6.5% as the cutoff indicating diabetes. We counted the cumulative frequency distribution of measured HbA_{1c} levels by diabetes status, then re-estimated the frequency distribution after regression analysis adjusting for the seven SNPs at the nonglycemic loci, recalibrating the distribution to have the same mean HbA_{1c} as in each original cohort. We counted the proportion of undiagnosed diabetic individuals with unadjusted HbA_{1c} ≥6.5% who had an adjusted HbA_{1c} <6.5%, and the proportion of nondiabetic individuals with unadjusted HbA_{1c} <6.5% who had an adjusted HbA_{1c} ≥6.5%. The difference between these proportions is called "net reclassification" and in this instance indicates the overall proportion of a population whose diagnostic status would change based on the influence of these seven common, nonglycemic genetic variants.

RESULTS

New common variants associated with HbA_{1c}. We carried out a meta-analysis of SNP associations with HbA_{1c} levels in up to 46,368 participants of European ancestry from 31 cohorts. We identified 10 genomic regions associated with HbA_{1c} levels (Table 2, Figs. 1 and 2). Six associated regions were new, including *FN3K* (rs1046896, $P = 1.57 \times 10^{-26}$), *HFE* (rs1800562, $P = 2.59 \times 10^{-20}$), *TMPRSS6* (rs855791, $P = 2.74 \times 10^{-4}$), *ANK1* (rs4737009, $P = 6.11 \times 10^{-12}$), *SPTA1* (rs2779116, $P = 2.75 \times 10^{-9}$), and *ATP11A/TUBGCP3* (rs7998202, $P = 5.24 \times 10^{-9}$). A second, independent SNP near *ANK1* was also associated with HbA_{1c} (rs6474359, $P = 1.18 \times 10^{-8}$; r^2 with rs4737009 = 0.0001; see also supplementary METHODS). In addition, SNPs in or near *HK1* (rs16926246, $P = 3.11 \times 10^{-54}$), *MTNR1B* (rs1387153, $P = 3.96 \times 10^{-11}$), *GCK* (rs1799884, $P = 1.45 \times 10^{-20}$), and *G6PC2/ABCB11* (rs552976, $P = 8.16 \times 10^{-18}$) were associated with HbA_{1c} levels. These loci had previously been associated with HbA_{1c} (15,16), FG (9–12,14,15) and/or type 2 diabetes risk (9–12,15,16,19). Associations were generally similar across cohorts, showing no significant heterogeneity (Table 2). This lack of heterogeneity suggests that there is good consistency in trait measurement across different cohorts.

Pleiotropy and mediation of SNP-HbA_{1c} associations. HbA_{1c} levels are influenced by average ambient glycemia over the preceding 3 months, and possibly by erythrocyte turnover. We therefore investigated the novel HbA_{1c} loci for associations with several diabetes-related and hematologic quantitative parameters in the MAGIC cohorts (19,24) (supplementary Table S4). As previously shown (19), 3 of 10 loci, *GCK*, *MTNR1B*, and *G6PC2*, were associated with FG and HOMA-B (an index of β-cell function, Table 3 and supplementary Table S3), and *GCK* was additionally associated with 2-h glucose. In all cases, the allele associated with increased HbA_{1c} was also associated with increased FG and 2-h glucose. No HbA_{1c}-associated SNP was significantly associated with measures of insulin (supplementary Table S3). We further used conditional models to investigate whether FG levels mediated associations of SNPs with HbA_{1c}. In these analyses a marked attenuation of the effect size of the SNP in a model adjusted for FG compared with the original main effects model would be consistent with the hypothesis that glycemic pathways primarily account for, or mediate, the HbA_{1c} association. For the three loci associated with FG (*GCK*, *MTNR1B*, and *G6PC2/ABCB11*), effect sizes were

TABLE 2
Associations with HbA_{1C} of 10 independent loci identified in the meta-analysis

SNP	Chr	Pos (B36)	Nearest locus	Effect/ other allele	CEU freq (effect)	HbA_{1C} (%) association				Heterogeneity		
						Freq (effect)	*N*	β (SE)	*P*	χ^2 *P* value	*Q P*	I^2 (%)
rs2779116	1	156,852,039	*SPTA1*	T/C	0.32	0.27	34,663	0.024 (0.004)	2.75×10^{-9}	0.673	0.606	0
rs552976	2	169,616,945	*G6PC2/ ABCB11*	G/A	0.66	0.64	40,420*	0.047 (0.003)	8.16×10^{-18}	0.596	0.591	0
rs1800562	6	26,201,120	*HFE*	G/A	0.96	0.94	43,778*	0.063 (0.007)	2.59×10^{-20}	0.661	0.300	11
rs1799884	7	44,002,308	*GCK*	T/C	0.20	0.18	45,591*	0.038 (0.004)	1.45×10^{-20}	0.187	0.120	24
rs6474359	8	41,668,351	*ANK1*	T/C	0.97	0.97	29,997	0.058 (0.011)	1.18×10^{-8}	0.328	0.267	15
rs4737009	8	41,749,562	*ANK1*	A/G	0.28	0.24	36,862	0.027 (0.004)	6.11×10^{-12}	0.182	0.182	21
rs16926246	10	70,763,398	*HK1*	C/T	0.89	0.90	42,707*	0.089 (0.004)	3.11×10^{-54}	0.329	0.162	21
rs1387153	11	92,313,476	*MTNR1B*	T/C	0.28	0.28	32,293	0.028 (0.004)	3.96×10^{-11}	0.867	0.857	0
rs7998202	13	112,379,869	*ATP11A/ TUBGCP3*	G/A	0.15	0.14	34,724	0.031 (0.005)	5.24×10^{-9}	0.415	0.383	6
rs1046896	17	78,278,822	*FN3K*	T/C	0.25	0.31	45,953*	0.035 (0.003)	1.57×10^{-26}	0.450	0.440	2
rs855791	22	35,792,882	*TMPRSS6*	A/G	0.39	0.42	34,562	0.027 (0.004)	2.74×10^{-14}	0.970	0.962	0

*Indicates SNPs for which additional de novo genotyping was performed in eight cohorts. The β coefficient denotes the per-effect allele increase in HbA_{1C} (%) at that locus.

substantially decreased in FG-conditioned models, whereas at the other seven loci, effect sizes remained essentially unchanged (Table 3), indicating that associations with HbA_{1c} at these loci are unlikely to be mediated by glycemic factors.

We also investigated associations of HbA_{1c} loci with several hematologic parameters in a subset of four populations with available data (KORA F3, KORA F4, SardiNIA, and NHANES III, supplementary Table S3). Two HbA_{1c} loci (encoding for functional alleles at *HFE* and *TMPRSS6*) showed genome-wide significant association with erythrocyte indexes, consistent with an influence of erythrocyte physiology on HbA_{1c} variability. The HbA_{1c}-raising alleles had diverse effects, including associations with lower hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and iron, and higher transferrin (*HFE* and *TMPRSS6*). In addition, three loci (*SPTA1*, *ANK1*, and *HK1*) showed suggestive associations ($P < 5 \times 10^{-3}$) with erythrocyte indexes, with HbA_{1c}-raising alleles associated with increased MCV (*SPTA1*, *ANK1*), or lower hemoglobin (*HK1*).

We used these same four cohorts where those parameters were available to carry out a meta-analysis on HbA_{1c} levels, this time conditioning for the hematologic traits. We did not observe any difference at the three "glycemic" loci, although attenuation of β estimates was observed at *HFE*, *TMPRSS6*, and *HK1* (supplementary Table S4). However, the sample size used for this analysis was relatively underpowered, resulting in nonsignificant differences (*P* value > 0.1) and we lacked power for other loci, indicating the need for future analysis in larger sample collections.

Associations with disease: type 2 diabetes and CAD risk. HbA_{1c} has been shown to have strong epidemiologic associations with type 2 diabetes risk and with CAD risk in persons without diabetes. To ascertain if the novel loci affected type 2 diabetes risk, we tested associations in well-powered datasets. In a previous meta-analysis of 40,655 type 2 diabetes cases and 87,022 controls in MAGIC (19), *MTNRB1*, and *GCK* showed significant evidence of association (rs1387153 OR = 1.09, 95% CI 1.06–1.12, $P = 8.0 \times 10^{-13}$; rs1799884 OR = 1.07, 95% CI 1.05–1.10, $P = 5.0 \times 10^{-8}$), whereas *G6PC2/ABCB11* did not (rs552976 OR = 0.97, 95% CI 0.95–0.99, $P = 0.012$). We tested the other novel loci reported here for associations with type 2 diabetes in a partly overlapping study of 8,130 cases and 38,987 controls from the DIAGRAM+ consortium (22) (supplementary Table S3). No other locus associated with HbA_{1c} was associated with type 2 diabetes risk.

We also tested for associations with CAD using data from nine case/control studies of European descent (13,925 cases and 14,590 controls, supplementary Table S5). None of the SNPs associated with HbA_{1c} were associated with CAD in the combined sample of 28,515 participants (supplementary Table S6).

Effect size estimates for HbA_{1c}-associated loci. In a regression model, the 10 loci combined explained ~2.4% of the total variance in HbA_{1c} levels, or about 5% of estimated HbA_{1c} heritability. We calculated a genotype score using four of the largest population-based studies (ARIC, SardiNIA, KORA F4, and FHS). Using the 10 HbA_{1c} loci, we estimated cohort-specific differences between the top and bottom 10% of the genotype score distribution (mean [SE] % HbA_{1c}) to be: 5.25% (0.01) and 5.50% (0.004), respectively ($P = 3.61 \times 10^{-33}$) for ARIC; 5.37% (0.027) and 5.49% (0.027) ($P = 1.36 \times 10^{-3}$) for SardiNIA; 5.32% (0.024) and 5.58% (0.027) ($P = 4.64 \times 10^{-12}$) for KORA F4; and 5.07% (0.046) and 5.38% (0.046) ($P = 1.45 \times 10^{-6}$) for FHS. The corresponding weighted average difference between the top and bottom 10% of the HbA_{1c} distributions was 0.21%. For a genotype score using only the seven nonglycemic loci (*FN3K*, *HFE*, *TMPRSS6*, *ANK1*, *SPTA1*, *ATP11A/TUBGCP3*, and *HK1*), the weighted average difference between the top and bottom 10% of the HbA_{1c} distributions was 0.19%.

Net reclassification in diabetes screening with HbA_{1c}. We used net reclassification analysis to estimate the population-level impact of the seven nonglycemic loci when HbA_{1c} ≥6.5 (%) is used as the reference cutoff for diabetes diagnosis, as recently proposed (18). We calculated the net reclassification around this threshold attributable to effects of the seven nonglycemic HbA_{1c} loci that might be expected when screening a general European ancestry population for undiagnosed diabetes using HbA_{1c}. We studied the FHS and ARIC cohorts combined ($N = 10,110$), and included individuals with undiagnosed diabetes for detection by screening. We compared the

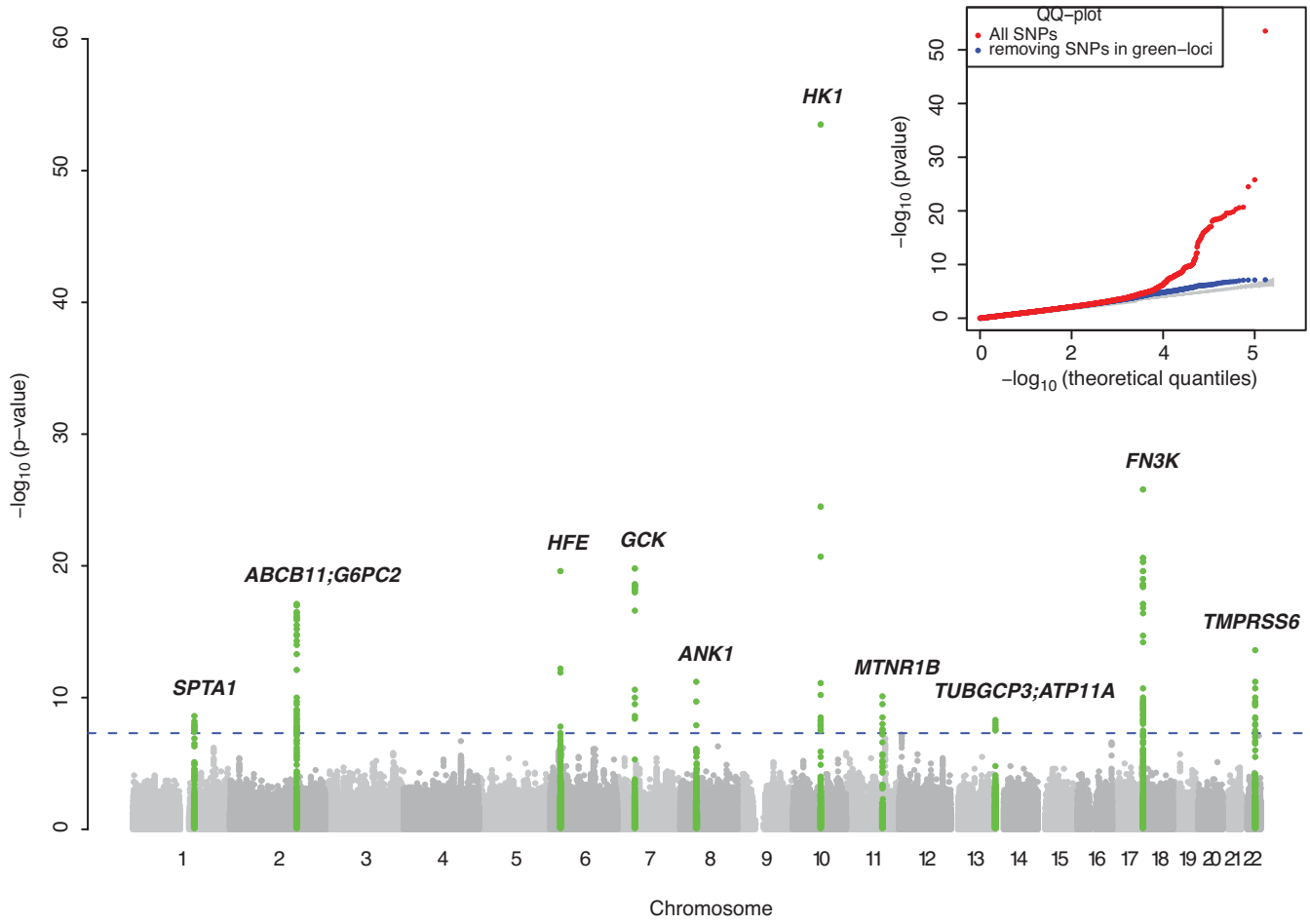


FIG. 1. Manhattan plot and quantile-quantile (QQ) plot of association findings. The figure summarizes the genome-wide association scan results combined across all studies by inverse variance weighting. The blue dotted line marks the threshold for genome-wide significance (5×10^{-8}). SNPs in loci exceeding this threshold are highlighted in green. A QQ plot is shown in the inset panel, where the red line corresponds to all test statistics, and the blue line to results after excluding statistics at all associated loci (highlighted in green in the Manhattan plot). The gray area corresponds to the 90% confidence region from a null distribution of *P* values (generated from 100 simulations). (A high-quality color representation of this figure is available in the online issue.)

measured distribution of HbA_{1c} to the distribution adjusted for the seven nonglycemic SNPs (Fig. 3). The net reclassification was −1.86% ($P = 0.002$), indicating that the population-level effect size of the 7 nonglycemic HbA_{1c}-associated SNPs is equivalent to reclassification of about 2% of an European ancestry population sample according to HbA_{1c}-determined diabetes status.

DISCUSSION

HbA_{1c} levels are influenced by ambient glycemia, and also by erythrocyte biology, as seen in hereditary anemias and iron storage disorders caused by rare, highly-penetrant genetic variants. We analyzed associations of HbA_{1c} levels with common genetic variants associated in a meta-analysis of up to 46,000 nondiabetic individuals of European descent from 31 cohorts. We identified 10 loci associated with HbA_{1c} at genome-wide levels of significance, with 1 locus, *ANK1*, showing 2 independent signals. Of these, six (in or near *FN3K*, *HFE*, *TMPRSS6*, *ATP11A/TUBGCP3*, *ANK1*, and *SPTA1*) represent new common genetic determinants of HbA_{1c}, and four (*GCK*, *G6PC2/ABCB11*, *MTNR1B*, and *HK1*) are confirmatory (9–11; 13–16; and 25).

Fasting and postprandial glucose levels are key determinants of HbA_{1c}. Of the 10 loci identified, those in *GCK*, *G6PC2*, and *MTNR1B* were strongly associated with levels of FG in this and previous studies (8; 10; 12–16; 19). Two of them (*GCK* and *MTNR1B*) were also associated with type 2 diabetes (19). Analyses conditioned on FG further supported an effect on HbA_{1c} via regulation of systemic glucose concentrations for *GCK*, *G6PC2*, and *MTNR1B* loci alone. No other HbA_{1c} locus was associated with type 2 diabetes risk or quantitative type 2 diabetes risk factors, suggesting that associations with HbA_{1c} levels were not likely to be mediated by ambient glycemia. Rare variants at some of these loci (*HK1*, encoding hexokinase 1; *ANK1*, ankyrin; *SPTA1*, spectrin) cause hereditary anemias, and common variants at some loci are associated with quantitative hematologic traits as well as HbA_{1c} (25,26). This is consistent with the hypothesis that these common variants influence HbA_{1c} levels via erythrocyte physiology. Specific mechanisms are suggested by existing knowledge on the function of leading candidate genes in each region (see the supplemental on-line appendix).

HK1 is a good example to consider mechanism of action of common variants, as it has confirmed support as a true-positive HbA_{1c}-associated locus (16,27) and rare variants in *HK1* are associated with nonspherocytic hemolytic anemia (MIM 142600) (28,29). *HK1* encodes the erythrocyte isoform of hexokinase, which determines the intra-

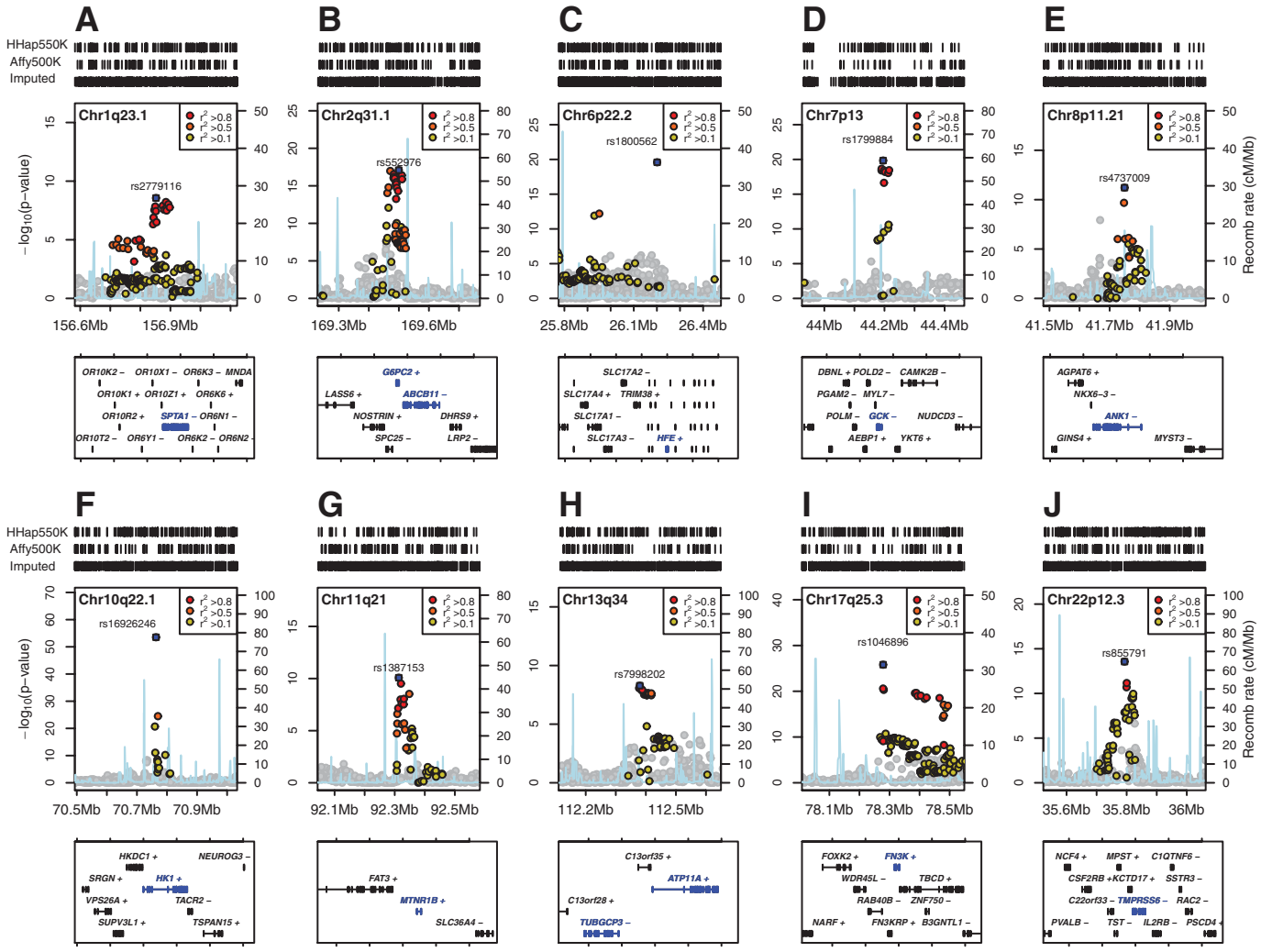


FIG. 2. Regional association plots at the HbA1c loci. Each panel spans ± 250 kb around the most significant associated SNP in the region, which is highlighted with a blue square (panel *C* spans ± 300 kb). At the top of each panel, comb diagrams indicate the location of SNPs in the Illumina HumanHap 550K and Affymetrix 500K chips, and of SNPs imputed. The SNPs are colored according to their linkage disequilibrium with the top variant based on the CEU HapMap population (http://www.hapmap.org). Gene transcripts are annotated in the lower box, with the most likely biologic candidate highlighted in blue; ± indicates the direction of transcription. In panel *C*, a few gene names were omitted for clarity. Here, genes are, from left to right, *SCGN*, *HIST1H2AA*, *HIST1H2BA*, *SLC17A4*, *SLC17A1*, *SLC17A3*, *SLC17A2*, *TRIM38*, *HIST1H1A*, *HIST1H3A*, *HIST1H4A*, *HIST1H4B*, *HIST1H3B*, *HIST1H2AB*, *HIST1H2BB*, *HIST1H3C*, *HIST1H1C*, *HFE*, *HIST1H4C*, *HIST1H1T*, *HIST1H2BC*, *HIST1H2AC*, *HIST1H1E*, *HIST1H2BD*, *HIST1H2BD*, *HIST1H2BE*, *HIST1H4D*, *HIST1H3D*, *HIST1H2AD*, *HIST1H2BF*, *HIST1H4E*, *HIST1H2BG*, *HIST1H2AE*, *HIST1H3E*, *HIST1H1D*, *HIST1H4F*, *HIST1H4G*, *HIST1H3F*, *HIST1H2BH*, *HIST1H3G*, *HIST1H2BI*, and *HIST1H4H*. In panel *D*, the names of the first two genes, *UBE2D4* and *WBSCR19*, were also omitted for clarity. (A high-quality color representation of this figure is available in the online issue.)

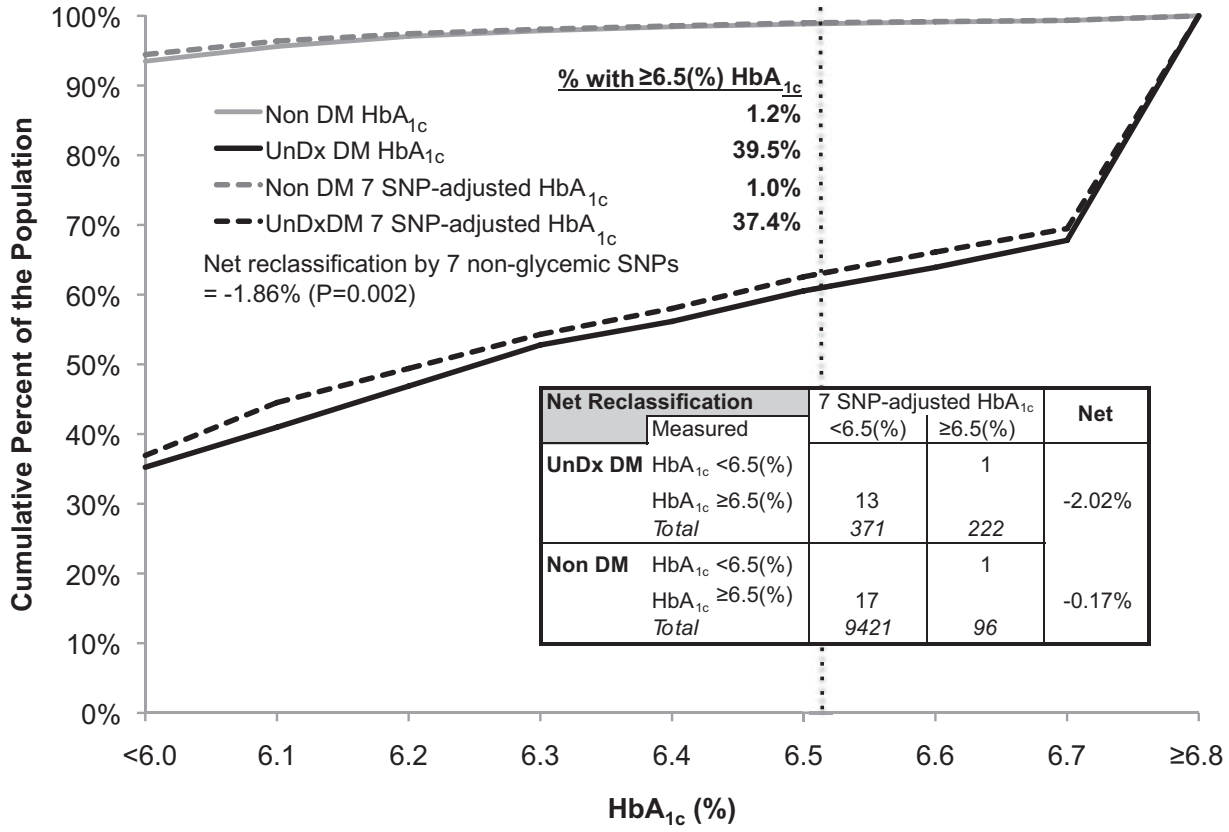
cellular commitment of glucose to the glycolytic pathway by catalyzing the conversion of intracellular glucose to glucose-6-phosphate. One plausible explanation for the observed association lies in the potential dissociation between ambient plasma glucose and intracellular cytoplasmic glucose that might be induced by functional variants at *HK1*; since the enzyme is preferentially active in erythrocytes, the intracellular utilization (metabolism) of glucose may not be reflective of systemic levels of glycemia. In support of this notion, the HbA_{1c}-raising allele was not associated with any glycemic traits in another recent study of European cohorts, but had robust associations with lower hemoglobin and hematocrit (27). In the CHARGE consortium, common variants in *HK1* were associated with decreased hemoglobin (25). We postulate, therefore, that the hemoglobin-lowering variant may affect the overall percentage of HbA_{1c} through an increased glucose/hemoglobin molar ratio, which in turn could increase the rate of hemoglobin that is glycated at a given glucose level. Variation in rates of deglycation and of erythrocyte turnover also are likely to play an important role in measured HbA_{1c} levels. These hypotheses require further testing. A possible role of erythrocyte membrane stability and altered erythrocyte life span (*ANK1*, *SPTA1*) and hemoglobin deglycation (*FN3K*) may be postulated based on the known function of the respective gene products (supplementary online appendix).

A role for iron homeostasis influencing HbA_{1c} is suggested by the *HFE* and *TMPRSS6* loci, where associations were observed at known functional variants in two complementary and directionally consistent pathways (30). At *HFE* the A allele at rs1800562 (Cys262Tyr), which is responsible for hereditary hemochromatosis (MIM 235200), was associated with *lower* levels of HbA_{1c}, rather than the higher levels one would predict from epidemiologic observations of the increased *HFE* mutation preva-

TABLE 3
Associations with HbA_{1C} of 10 independent loci conditioned on levels of fasting or 2-h glucose

				Fasting glucose			2-h glucose		
SNP	Nearest locus	Effect/ Other		HbA_{1C} (%) adjusted for sex, age	HbA_{1C} (%) adjusted for glucose, sex, age	Fasting glucose (mmol/l) adjusted for sex, age	HbA_{1C} (%) adjusted for sex. age	HbA_{1C} (%) adjusted for 2 h-glucose, sex, age	2-h glucose (mmol/l) adjusted for sex, age
rs2779116	*SPTA1*	T/C	β (SE)	0.019 (0.004)	0.017 (0.004)	−0.001 (0.005)	0.026 (0.008)	0.029 (0.008)	0.029 (0.037)
			P	2.4×10^{-6}	1.7×10^{-6}	0.900	9.6×10^{-4}	2.9×10^{-4}	0.432
			N	20,700	21,359	21,505	6,394	6,347	6,347
rs552976	*G6PC2 /ABCB11*	G/A	β (SE)	0.028 (0.004)	0.013 (0.003)	0.060 (0.005)	0.029 (0.007)	0.027 (0.007)	−0.021 (0.034)
			P	4.5×10^{-15}	2.0×10^{-5}	6.3×10^{-36}	6.1×10^{-5}	1.4×10^{-4}	0.538
			N	23,496	23,496	23,642	6,393	6,346	6,346
rs1800562	*HFE*	G/A	β (SE)	0.054 (0.007)	0.048 (0.006)	−0.008 (0.010)	0.095 (0.016)	0.096 (0.016)	0.086 (0.073)
			P	3.1×10^{-13}	2.5×10^{-14}	0.419	1.1×10^{-9}	1.1×10^{-9}	0.239
			N	23,503	23,503	23,649	6,389	6,342	6,342
rs1799884	*GCK*	T/C	β (SE)	0.030 (0.005)	0.018 (0.004)	0.053 (0.0063)	0.037 (0.010)	0.039 (0.010)	0.111 (0.046)
			P	5.6×10^{-11}	7.3×10^{-6}	4.7×10^{-17}	1.3×10^{-4}	6.2×10^{-5}	0.0143
			N	23,497	23,497	23,643	6,394	6,347	6,347
rs4737009	*ANK1*	A/G	β (SE)	0.023 (0.004)	0.017 (0.004)	0.010 (0.006)	0.023 (0.008)	0.025 (0.008)	−0.049 (0.038)
			P	3.2×10^{-8}	2.7×10^{-6}	0.072	4.4×10^{-3}	2.3×10^{-3}	0.197
			N	21,355	21,355	21,501	6,390	6,343	6,343
rs16926246	*HK1*	C/T	β (SE)	0.073 (0.007)	0.069 (0.006)	−0.013 (0.009)	0.010 (0.017)	0.097 (0.017)	0.012 (0.092)
			P	4.8×10^{-26}	6.4×10^{-30}	0.178	1.6×10^{-9}	1.3×10^{-8}	0.899
			N	22,404	22,404	22,550	5,301	5,254	5,254
rs1387153	*MTNR1B*	T/C	β (SE)	0.027 (0.004)	0.013 (0.004)	0.056 (0.006)	0.035 (0.008)	0.032 (0.009)	0.036 (0.040)
			P	1.9×10^{-11}	2.2×10^{-4}	1.8×10^{-23}	3.1×10^{-5}	1.5×10^{-4}	0.362
			N	20,162	20,162	20,308	6,394	6,347	6,347
rs7998202	*ATP11A/TUBGCP3*	G/A	β (SE)	0.027 (0.006)	0.023 (0.005)	0.013 (0.008)	0.041 (0.012)	0.035 (0.012)	−0.035 (0.054)
			P	3.4×10^{-6}	1.3×10^{-5}	0.108	4.0×10^{-4}	2.6×10^{-3}	0.512
			N	21,359	21,359	21,505	6,394	6,347	6,347
rs1046896	*FN3K*	T/C	β (SE)	0.030 (0.004)	0.026 (0.003)	0.005 (0.005)	0.045 (0.008)	0.043 (0.008)	−0.011 (0.036)
			P	2.0×10^{-16}	1.0×10^{-15}	0.343	3.0×10^{-9}	2.0×10^{-8}	0.753
			N	23,496	23,496	23,642	6,393	6,346	6,346
rs855791	*TMPRSS6*	A/G	β (SE)	0.020 (0.004)	0.019 (0.003)	−0.006 (0.005)	0.024 (0.008)	0.022 (0.008)	0.009 (0.036)
			P	6.7×10^{-8}	8.3×10^{-9}	0.223	1.7×10^{-3}	6.1×10^{-3}	0.815
			N	23,508	23,508	23,654	6,394	6,347	6,347

β (SE) is the per-effect allele increase in HbA_{1C} (%) as in Table 2. For analyses conditional on fasting glucose, data were available for up to 23,654 samples from 15 cohorts (ARIC, BLSA, CROATIA, Fenland, FHS, DESIR, GENOMEUTWIN, Lolipop, NTR, ORCADES, SardiNIA, KORA F4, DGI, Sorbs and Health2000). For analyses conditional on 2-h glucose, data were available for only a smaller set of six cohorts totaling up to 6,394 samples (BLSA, Fenland, FHS, KORA F4, DGI and Sorbs). The SNP association with HbA_{1C} after adjusting for fasting glucose is attenuated most at the *G6PC2/ABCB11*, *GCK* and *MTNR1B* loci. Associations at *ANK1* are given for rs4737009, with the *ANK1* SNP showing the strongest association with HbA_{1C}.



Net Reclassification	Measured	7 SNP-adjusted HbA_{1c} <6.5(%)	7 SNP-adjusted HbA_{1c} ≥6.5(%)	Net
UnDx DM	HbA_{1c} <6.5(%)		1	
	HbA_{1c} ≥6.5(%)	13		-2.02%
	Total	*371*	*222*	
Non DM	HbA_{1c} <6.5(%)		1	
	HbA_{1c} ≥6.5(%)	17		-0.17%
	Total	*9421*	*96*	

FIG. 3. Net reclassification when screening for undiagnosed diabetes, using HbA1c as a population-level measure of genetic effect size. The figure shows the distribution of HbA1c in the FHS and ARIC cohorts combined (N = 10,110), stratified by individuals with undiagnosed type 2 diabetes (UnDx DM, N = 593, *black lines*) or without diabetes (Non DM, N = 9,517, *gray lines*), and by HbA1c without adjustment (*solid lines*) or after adjustment for seven nonglycemic SNPs (*dashed lines*). The *vertical dashed line* is the diabetes diagnostic threshold at HbA1c ≥6.5(%). Net reclassification is the overall proportion of the population appropriately moved above or below this line by considering the genetic information. For instance, among individuals with undiagnosed diabetes, 39.5% had an unadjusted HbA1c level ≥6.5 (%) and 37.4% had a seven SNP-adjusted HbA1c level ≥6.5 (%), and among those with undiagnosed diabetes, 2.02% of those with undiagnosed diabetes were misclassified by the influence of the seven SNPs. The net reclassification is calculated as the difference −2.02% − (−0.17%) = −1.86%.

lence in patients with type 2 diabetes (31,32). This apparently paradoxical relationship may be due to a shift in glucose to hemoglobin molar ratio associated with higher overall hemoglobin (supplementary Table S3), leading to consequent decrease in the percentage of glycated hemoglobin. The reciprocal observation is seen for *TMPRSS6*, where the A allele at SNP rs855791 (Val736Ala) was associated with lower hemoglobin levels and *higher* HbA_{1c} levels, as one would predict in a state of iron deficiency and disproportionately lower total hemoglobin concentrations.

It is known that conditions characterized by altered erythrocyte physiology may influence the utility of HbA_{1c} in diabetes diagnosis (2–4,18), although this has generally been attributed to specific pathologies, such as inherited hemoglobinopathies, rather than to physiologic variation in the general population. We show here for the first time that the common genetic variation resulting in subtler but more widespread alteration of iron levels or hemoglobin concentration can also affect HbA_{1c} levels. The absolute size of the genetic effect of 7 to 10 common SNPs associated with HbA_{1c} is about 0.2%, comparing the extremes of the HbA_{1c}-raising allele distribution. This is smaller than the 0.5% HbA_{1c} average intralaboratory variation for HbA_{1c}-certified labs reported as of 2000 (33). We sought to frame these genetic effects in population-level terms by comparing HbA_{1c} distributions without and with adjustment for the seven nonglycemic SNPs and calculating net reclassification around the 6.5% HbA_{1c} diagnostic threshold. We found the overall effect of the nonglycemic loci identified in this study to be small but detectable, potentially affecting about 2% of white individuals likely reclassified by diabetes status. This estimate represents an upper boundary for the effect of these common variants, as most people (the majority in the center of the distribution) are expected to have a smaller individual genotype effect size.

Our findings are therefore directly relevant to recent initiatives to focus diabetes diagnosis and care more centrally on HbA_{1c}. Although the 10 loci described here likely represent the strongest common association signals found in Europeans, they account for a relatively small proportion of total variance of HbA_{1c} and have minimal effect on diagnosis or misclassification of diabetes. Therefore, our study achieves a significant result in quantifying, for the first time, the misclassification risk associated with the top tier of HbA_{1c}-associated common genetic variation. Future research will be required to explore two main areas not addressed in this study. First, genetic association studies in diabetic individuals will be important to assess the contribution of HbA_{1c}-associated variants to its application in diabetes control. These analyses require different study designs to ours, and are beyond the scope of current datasets. Second, it will be important to explore associations of HbA_{1c} with low to intermediate frequency variants through imputa-

tion from the 1,000 Genomes Project, direct association using whole-genome sequencing data, and in-depth replication and locus fine-mapping through custom arrays.

Finally, it will be important to evaluate reclassification rates in different populations, because the allele frequencies of some SNPs shown to be associated with HbA_{1c} are known to vary substantially among populations with different ethnic ancestries. For instance, the A allele frequency at rs1800562 (*HFE*) in populations of European ancestry is 5% (CEU), but the A allele is absent in populations of African or East Asian ancestry (YRI, CHB/JPT). The T allele frequency at rs855791 (*TMPRSS6*) is 39% in CEU samples, but only 11 and 5% in the YRI and CHB/JPT samples, respectively. It will therefore be important to assess how variation in frequency and effect size influence the impact of HbA_{1c}-associated variants in diverse populations.

In summary, in a meta-analysis of GWAS in a large number of individuals of European ancestry, we identified 10 common genetic loci associated with HbA_{1c} levels. Six of these loci are novel, and seven appear to influence HbA_{1c} via nonglycemic erythrocyte and iron biologic pathways. The genetic effect size of this set of loci on variations in HbA_{1c} levels is small, but carries a detectable reclassification risk that will need to be refined by the discovery of additional variants and testing in diverse ancestral populations.

URLs. METAL, http://www.sph.umich.edu/csg/abecasis/Metal/index.html; HapMap, http://www.hapmap.org; R-project, http://www.r-project.org; 1,000 Genomes Project, http://www.1000genomes.org.

ACKNOWLEDGMENTS

Disclosures are listed in the online appendix.

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Acknowledgments are listed in the online appendix.

APPENDIX

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