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

Citation for published version:

Digital Object Identifier (DOI):
10.2337/db10-0502

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Early version, also known as pre-print

Published In:
Diabetes
Common Variants at 10 Genomic Loci Influence Hemoglobin A1c Levels via Glycemic and Nonglycemic Pathways

Nicolle Soranzo,1,2 Serena Sanna,3 Eleanor Wheeler,1 Christian Gieger,4 Dörte Radke,5 Josée Dupuis,6,7 Nabila Bouatia-Naji,8 Claudia Langenberg,9 Inga Prokopenko,10,11 Elliot Stolerman,12,13,14 Manjinder S. Sandhu,9,15,16 Matthew M. Heeney,17 Joseph M. Devaney,18 Muredach P. Reilly,19,20 Sally L. Ricketts,15 et al.*

OBJECTIVE—Glycated hemoglobin (HbA1c), 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 HbA1c. We aimed to identify such genetic factors and investigate the extent to which they influence diabetes classification based on HbA1c levels.

RESEARCH DESIGN AND METHODS—We studied associations with HbA1c 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 HbA1c 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 HbA1c, including six new loci near FN3K (lead SNP/P value, rs1046896/P = 1.6 × 10−20), HFE (rs1800562/P = 2.6 × 10−20), TMPRSS6 (rs855791/P = 2.7 × 10−14), ANK1 (rs4773009/P = 6.1 × 10−13), SP1A1 (rs2779116/P = 2.8 × 10−12) and ATP11A/TUBGCP3 (rs9898202/P = 5.2 × 10−9), and four known HbA1c loci: HK1 (rs16926246/P = 3.1 × 10−5), MTNR1B (rs1387153/P = 4.0 × 10−11), GCK (rs1799884/P = 1.5 × 10−28) and G6PC2/ABC11 (rs652976/P = 8.2 × 10−18). We showed that associations with HbA1c 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% HbA1c difference between the extreme 10% tails of the risk score, and would reclassify ~2% of a general white population screened for diabetes with HbA1c.

CONCLUSIONS—GWAS identified 10 genetic loci reproducibly associated with HbA1c. 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 HbA1c levels via erythrocyte biology, and confer a small but detectable reclassification of diabetes diagnosis by HbA1c. Diabetes 59:3229–3239, 2010

Glycated hemoglobin (HbA1c) 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 HbA1c 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 HbA1c in populations (2–4).

Common genetic variation also influences HbA1c variability. The heritability of HbA1c 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 HbA1c levels (7–15). A GWAS for HbA1c levels in 14,618 nondiabetic women found a suggestive association (P = 9.8 × 10−8) with SLC30A8 (a known type 2 diabetes locus) and genome-wide significant association (P < 5 × 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 HbA1c, but a more thorough accounting of common variants comprising the genetic architecture of HbA1c is needed.

In this study we tested the hypothesis that additional common genetic factors are associated with HbA1c. 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 HbA1c levels, we sought to place the size of the effect of novel genetic findings into the
population perspective of diabetes screening and diagnosis. HbA1c levels have recently been recommended for this use based on high overlap between HbA1c 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 HbA1c-associated loci shifted the population level distribution of HbA1c and thereby influenced diabetes screening using HbA1c.

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 a fasting glucose concentration ≥7.0 mmol/l. HbA1c (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 HbA1c 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 5%. For 5 SNPs (rs1046896, rs16926246, rs1799884, rs1800562, and rs11011164), we obtained further data from 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 included in the frequency analysis. We used a meta-analysis approach to calculate the proportion of variance explained by each SNP. The proportion of variance explained by each SNP was calculated as the ratio of the observed effect size to the total effect size across all studies. The proportion of variance explained by each SNP was calculated as the ratio of the observed effect size to the total effect size across all studies. The proportion of variance explained by each SNP was calculated as the ratio of the observed effect size to the total effect size across all studies. The proportion of variance explained by each SNP was calculated as the ratio of the observed effect size to the total effect size across all studies.
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 of meta-analysis.** In each cohort a linear regression model was fit using untransformed (percentage) HbA1c, as the dependent variable to evaluate the additive effect of genotyped and imputed SNPs. HbA1c 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 control lambda very close to the expected value of 1.0; thus, we report untransformed (percentage) HbA1c 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 lambda 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 chi-square 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 ANKI 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 Hba1c 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 Hba1c into two paths. The first path links the SNP directly to Hba1c, and the second path links the SNP to Hba1c through a mediator, e.g., FG or hematologic parameters. A marked attenuation of the size of effect on Hba1c of the SNP in the conditional “mediation” model implies that the SNP (e.g., rs92576) acts on the mediator (e.g., FG), which in turn acts on Hba1c 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 studies. Association statistics with type 2 diabetes were obtained from a previous analysis of the MAGIC datasets or from the DIAGRAM meta-analysis. Associations were tested in this way for Hba1c as 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 Hba1c-associated SNPs: 1) we used regression to estimate in percentages the total variance in Hba1c, 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 Hba1c (%) 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 Hba1c distributions at the population level.

**Net reclassification analysis.** Variation in the measured level of Hba1c associated with nonglycemic genetic effects may affect the classification of individuals as diabetic or nondiabetic when screening general population samples using Hba1c. We used this relationship as a way to understand the clinical influence of the Hba1c 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 Hba1c via primarily glycemic mechanisms (SPTA1, HFE, ANKI, HR1, ATP1A1/TUBGCP3, PNDK, and TMPRSS6), using the method of Pencina et al. (22). 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 Hba1c measured. We excluded as in previous analyses all individuals on diabetes treatment (diagnosed diabetes), but retained individuals with FG ≥7.8 mmol/l not on treatment (who we classified as having undiagnosed diabetes, N = 5935) as well as all nondiabetic individuals (N = 9,517). We then sought to differentiate these individuals on the basis of their Hba1c levels, using ≥6.5% as the cutoff indicating diabetes. We counted the cumulative frequency distribution of measured Hba1c levels by diabetes status, then re-estimated the frequency distribution after regression adjustment for the seven SNPs at the nonglycemic loci, recalibrating the distribution to have the same mean Hba1c as in each original cohort. We counted the proportion of undiagnosed diabetic individuals with unadjusted Hba1c ≥6.5% who had an adjusted Hba1c ≤6.5%, and the proportion of nondiabetic individuals with unadjusted Hba1c ≤6.5% who had an adjusted Hba1c ≥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 Hba1c.** We carried out a meta-analysis of SNP associations with Hba1c levels in up to 46,368 participants of European ancestry from 31 cohorts. We identified 10 genomic regions associated with Hba1c levels (Table 2, Figs. 1 and 2). Six associated regions were new, including FN3K (rs1046896, P = 1.57 × 10^{-2}), HFE (rs1800562, P = 2.59 × 10^{-20}), TMPRSS6 (rs855791, P = 2.74 × 10^{-4}), ANKI (rs4737009, P = 6.11 × 10^{-12}), SPTA1 (rs2779116, P = 2.75 × 10^{-9}), and ATP11A/TUBGCP3 (rs7998202, P = 5.24 × 10^{-8}). A second, independent SNP near ANKI was also associated with Hba1c (rs6474359, P = 1.18 × 10^{-8}; r^2 with rs4737009 = 0.0001; see also supplementary methods). In addition, SNPs in or near HKI (rs16926246, P = 3.11 × 10^{-54}), MTNR1B (rs1387153, P = 3.96 × 10^{-11}), GCK (rs1799884, P = 1.45 × 10^{-9}), and G6PC2/ABC2B1 (rs552976, P = 8.16 × 10^{-18}) were associated with Hba1c levels. These loci had previously been associated with Hba1c (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-Hba1c associations.** Hba1c levels are influenced by average ambient glycemia over the preceding 3 months, and possibly by erythrocyte turnover. We therefore investigated the novel Hba1c 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 Hba1c was also associated with increased FG and 2-h glucose. No Hba1c-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 Hba1c. 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 Hba1c association. For the three loci associated with FG (GCK, MTNR1B, and G6PC2/ABC2B1), effect sizes were
COMMON GENETIC VARIANTS AND HbA1c

substantially decreased in FG-conditioned models, this time conditioning for the hematologic traits. The HbA1c-raising erythrocyte indexes, consistent with an influence of erythrocye physiology on HbA1c variability. The HbA1c-raising erythrocyte indexes, consistent with an influence of erythrocye physiology on HbA1c variability. The HbA1c-raising erythrocyte indexes, consistent with an influence of erythrocye physiology on HbA1c variability. HbA1c (%). We used 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 HbA1c 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 HbA1c were associated with CAD in the combined sample of 28,515 participants (supplementary Table S6).

### Effect size estimates for HbA1c-associated loci

In a regression model, the 10 loci combined explained ~2.4% of the total variance in HbA1c levels, or about 5% of estimated HbA1c heritability. We calculated a genotype score using four of the largest population-based studies (ARIC, SardiNIA, KORA F4, and FHS). Using the 10 HbA1c loci, we estimated cohort-specific differences between the top and bottom 10% of the genotype score distribution (mean [SE] % HbA1c) to be: 5.25% (0.01) and 5.50% (0.004), respectively (P = 3.61 x 10^{-33}) for ARIC; 5.37% (0.027) and 5.49% (0.027) (P = 1.36 x 10^{-33}) for SardiNIA; 5.32% (0.024) and 5.58% (0.027) (P = 4.64 x 10^{-32}) for KORA F4; and 5.07% (0.046) and 5.38% (0.046) (P = 1.45 x 10^{-6}) for FHS. The corresponding weighted average difference between the top and bottom 10% of the HbA1c distributions was 0.21%. For a genotype score using only the seven nonglycemic loci (FN3K, HFE, TMPRSS6, ANKI, SPTA1, ATP11A/TUBGCP3, and HK1), the weighted average difference between the top and bottom 10% of the HbA1c distributions was 0.19%.

### Net reclassification in diabetes screening with HbA1c

We used net reclassification analysis to estimate the population-level impact of the seven nonglycemic loci when HbA1c ≥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 HbA1c loci that might be expected when screening a general European ancestry population for undiagnosed diabetes using HbA1c. We studied the FHS and ARIC cohorts combined (N = 10,110), and included individuals with undiagnosed diabetes for detection by screening. We compared the

### Table 2

Associations with HbA1c of 10 independent loci identified in the meta-analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Pos (B36)</th>
<th>Nearest locus</th>
<th>Effect/other allele</th>
<th>CEU freq (effect)</th>
<th>HbA1c (%) association</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2779116</td>
<td>1</td>
<td>156,852,039</td>
<td>SPTAI</td>
<td>C/T</td>
<td>0.32</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>rs552976</td>
<td>2</td>
<td>169,616,945</td>
<td>G6PC2/ABCB11</td>
<td>A/G</td>
<td>0.66</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>rs1808562</td>
<td>6</td>
<td>26,201,120</td>
<td>HFE</td>
<td>T/C</td>
<td>0.96</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>rs1799884</td>
<td>7</td>
<td>44,002,308</td>
<td>GCK</td>
<td>T/C</td>
<td>0.20</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>rs6474359</td>
<td>8</td>
<td>41,668,351</td>
<td>ANKI</td>
<td>T/C</td>
<td>0.97</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>rs4737009</td>
<td>8</td>
<td>41,749,562</td>
<td>HK1</td>
<td>C/T</td>
<td>0.89</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>rs16926246</td>
<td>10</td>
<td>70,763,398</td>
<td>HK1</td>
<td>T/C</td>
<td>0.28</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>rs1387153</td>
<td>11</td>
<td>92,313,476</td>
<td>MTNR1B</td>
<td>T/C</td>
<td>0.28</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>rs7998202</td>
<td>13</td>
<td>112,379,869</td>
<td>ATP11A/TUBGCP3</td>
<td>G/A</td>
<td>0.15</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>rs1046986</td>
<td>17</td>
<td>78,278,822</td>
<td>FN3K</td>
<td>T/C</td>
<td>0.25</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>rs855791</td>
<td>22</td>
<td>35,792,882</td>
<td>TMPRSS6</td>
<td>A/G</td>
<td>0.39</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

*a*Indicates SNPs for which additional de novo genotyping was performed in eight cohorts. The β coefficient denotes the per-allele increase in HbA1c (%) at that locus.
measured distribution of HbA1c to the distribution adjusted for the seven nonglycemic SNPs (Fig. 3). The net reclassification was $\frac{1.86\%}{P = 0.002}$, indicating that the population-level effect size of the 7 nonglycemic HbA1c-associated SNPs is equivalent to reclassification of about 2% of an European ancestry population sample according to HbA1c-determined diabetes status.

**DISCUSSION**

HbA1c 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 HbA1c 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 HbA1c at genome-wide levels of significance, with 1 locus, $\text{ANK1}$, showing 2 independent signals. Of these, six (in or near $\text{FN3K, HFE, TMPRSS6, ATP11A/TUBGCP3, ANK1, and SPTA1}$) represent new common genetic determinants of HbA1c, and four ($\text{GCK, G6PC2/ABCB11, MTNR1B, and HK1}$) are confirmatory (9–11; 13–16; and 25).

Fasting and postprandial glucose levels are key determinants of HbA1c. Of the 10 loci identified, those in $\text{GCK}$, $\text{G6PC2}$, and $\text{MTNR1B}$ were strongly associated with levels of FG in this and previous studies (8; 10; 12–16; 19). Two of them ($\text{GCK}$ and $\text{MTNR1B}$) were also associated with type 2 diabetes (19). Analyses conditioned on FG further supported an effect on HbA1c via regulation of systemic glucose concentrations for $\text{GCK}$, $\text{G6PC2}$, and $\text{MTNR1B}$ loci alone. No other HbA1c locus was associated with type 2 diabetes risk or quantitative type 2 diabetes risk factors, suggesting that associations with HbA1c levels were not likely to be mediated by ambient glycemia. Rare variants at some of these loci ($\text{HK1}$, encoding hexokinase 1; $\text{ANK1}$, ankyrin; $\text{SPTA1}$, spectrin) cause hereditary anemias, and common variants at some loci are associated with quantitative hematologic traits as well as HbA1c (25,26). This is consistent with the hypothesis that these common variants influence HbA1c 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).

$\text{HK1}$ is a good example to consider mechanism of action of common variants, as it has confirmed support as a true-positive HbA1c-associated locus (16,27) and rare variants in $\text{HK1}$ are associated with nonspherocytic hemolytic anemia (MIM 142600) (28,29). $\text{HK1}$ encodes the erythrocyte isoform of hexokinase, which determines the intra-
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 HbA1c-raising allele was not associated with any glycemic traits in another recent study of European cohorts, but had robust associations with reduced hemoglobin (25). We postulate, therefore, that the hemoglobin-lowering variant may affect the overall percentage of HbA1c 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 glycation and of erythrocyte turnover also are likely to play an important role in measured HbA1c levels. These hypotheses require further testing. A possible role of erythrocyte membrane stability and altered erythrocyte life span (ANK1, SPTA1) and hemoglobin glycation (FN3K) may be postulated based on the known function of the respective gene products (supplementary online appendix).

A role for iron homeostasis influencing HbA1c 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 HbA1c, rather than the higher levels one would predict from epidemiologic observations of the increased HFE mutation prevalence.
**TABLE 3**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Age</th>
<th>Fasting Glucose</th>
<th>HbA1C (%) Adjusted for 2-h Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4737009</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1387153</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1799884</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2779116</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** HbA1C is expressed as the percentage of glycated hemoglobin. Associations with HbA1C (%) are given for selected loci, and associations with HbA1C (%) adjusted for fasting glucose are given for rs4737009, with the ANK1 locus.
It is known that conditions characterized by altered erythrocyte physiology may influence the utility of HbA1c concentrations. The reciprocal observation is seen for the top tier of HbA1c-associated common genetic variation. This is potentially affecting about 2% of white individuals likely represented 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 HbA1c. 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 HbA1c and have minimal effect on diagnosis or reclassification of diabetes. Therefore, our study achieves a significant result in quantifying, as one would predict in a state of iron deficiency and disproportionately lower total hemoglobin concentrations.

The 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 TM-PRSS6, where the A allele at SNP rs855791 (Val736Ala) was associated with lower hemoglobin levels and higher HbA1c 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 HbA1c 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, the misclassification risk associated with the common genetic variation resulting in subtler but more widespread alteration of iron levels or hemoglobin concentration can also affect HbA1c levels. The absolute size of the genetic effect of 7 to 10 common SNPs associated with HbA1c is about 0.2%, comparing the extremes of the HbA1c-raising allele distribution. This is smaller than the 0.5% HbA1c average intralaboratory variation for HbA1c-certified labs reported as of 2000 (33). We sought to frame these genetic effects in population-level terms by comparing HbA1c distributions without and with adjustment for the seven nonglycemic SNPs and calculating net reclassification around the 6.5% HbA1c 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 HbA1c. 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 HbA1c and have minimal effect on diagnosis or reclassification of diabetes. Therefore, our study achieves a significant result in quantifying, as one would predict in a state of iron deficiency and disproportionately lower total hemoglobin concentrations.
tion from the 1,000 Genomes Project, direct association using whole-genome sequencing data, and in-depth replication rates 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 HbA1c 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 HbA1c-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 HbA1c levels. Six of these loci are novel, and seven appear to influence HbA1c via nonglycemic erythrocyte and iron biologic pathways. The genetic effect size of this set of loci on variations in HbA1c 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.

ACKNOWLEDGMENTS

Disclosures are listed in the online appendix.

Parts of this study were presented in abstract form at the 70th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 25–29 June 2010.

Acknowledgments are listed in the online appendix.

APPENDIX

Nicole Soranzo,1,2 Serena Sanna,3 Eleanor Wheeler,1 Christian Gieger,4 Dörte Radke,9 Josée Dupuis,5,7 Nabila Bouatia-Naji,1 Claudia Langenberg,9 Inga Prokopenko,8,10,11 Elliot Stolerman,12,13,14 Manjinder S. Sandhu,8,15,16 Matthew M. Heaney,17 Joseph M. Devaney,18 Mureka P. Reilly,19,20 Sally L. Ricketts,15 Alexandre F.R. Stewart,21 Benjamin F. Voight,12,13,22 Christina Willenborg,23,24 Benjamin Wright,25 David Altshuler,12,13,14 Dan Arking,26 Beverley Balkau,27,28 Daniel Barnes,9 Eric Boerwinkle,29 Bernhard Böhm,30 Amélie Bonnefond,3 Lori L. Bonnycastle,31 Dorret I. Boomsma,22 Stefan R. Bornstein,33 Yvonne Böttcher,34 Suzannah Bumpstead,4 Mary Susan Burnett-Miller,35 Harry Campbell,35 Antonio Cao,3 John Chambers,36 Robert Clark,37 Francis S. Collins,31 Josef Coresh,38 ECO J.C. de Geus,39 Mariano Dei,3 Panos Deloukas,1 Angela Döring,1 Josephine M. Egan,39 Roberto Eloxe,4 Luigi Ferrucci,41 Nita Forouhi,9 Caroline S. Fox,7,42 Coresh,38 Eco J.C. de Geus,39 Mariano Dei,3 Panos Deloukas,1 Angela Döring,1 Josephine M. Egan,39 Roberto Eloxe,4 Luigi Ferrucci,41 Nita Forouhi,9 Caroline S. Fox,7,42

REFERENCES

glucokinase genes alters fasting glucose and birth weight: association in six studies and population-genetics analyses. Am J Hum Genet 2006;79:991–1001


COMMON GENETIC VARIANTS AND HbA1c


nonspherocytic hemolytic anemia. Blood 1983;61:12–18


Bij ASA, Ackermann JW, van den Wall Bake AW, Hofstede DP, Staat GE. Generalized hexokinase deficiency in the blood cells of a patient with nonsphero-}

nonspherocytic hemolytic anemia. Blood 1983;61:12–18

Bianchi M, Magnani M. Hexokinase mutations that produce nonsphero-}

nonspherocytic hemolytic anemia. Blood 1983;61:12–18

Generalized hexokinase deficiency in the blood cells of a patient with nonsphero-}

nonspherocytic hemolytic anemia. Blood 1983;61:12–18

Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Anderson NC. The transferrin receptor modulates Hfe-dependent regulation of hepcidin ex-}


