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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

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Download date: 13. Apr. 2022
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 F3N3K (lead SNP P value, rs1046896/P = 1.6 × 10$^{-26}$), HFE (rs1800562/P = 2.6 × 10$^{-29}$), TMPRSS6 (rs855791/P = 2.7 × 10$^{-14}$), ANK1 (rs4737009/P = 6.1 × 10$^{-15}$), SPTA1 (rs2779116/P = 2.8 × 10$^{-6}$) and ATP11A/TUBGCP3 (rs9353202/P = 5.2 × 10$^{-9}$), and four known HbA$_{1c}$ loci: HK1 (rs16026246/P = 3.1 × 10$^{-54}$), MTNRR1B (rs1387153/P = 4.0 × 10$^{-11}$), GCK (rs1799884/P = 1.5 × 10$^{-26}$) and G6PC2/Abci3 (rs529767/P = 8.2 × 10$^{-18}$). We show that associations with HbA$_{1c}$ are partly a function of hyperglycemia or blood transfusion, as well as genetic hereditary anemia 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).

CONCLUSIONS—GWAS identified 10 genetic loci reproducibly associated with HbA$_{1c}$. Six are novel and seven map to loci where rarer variants cause hereditary anemia 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

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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 an 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).
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 FG ≥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 SEs for 2-h glucose levels in males and females, respectively, and conditional models were run in KORA S4, a follow-up visit of KORA S4 samples. Mean and SEs for HbA1c levels in males and females, respectively, were: 5.66 (0.67)/5.60 (0.57) for HbA1C and 5.82 (1.20)/5.40 (1.01) for glucose. 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. (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), 30.31 (1.74)/29.52 (1.87) (SardiNIA) and 29.14 (3.60)/28.40 (3.69) (SardiNIA). The means for MCV (μmol/l, males/females) were 77.29 (9.28)/75.64 (9.22) (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. (SardiNIA) and 1.96 (0.52)/2.07 (0.57) (SardiNIA).
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 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 statistics 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 squared test implemented in METAL, Cochran’s Q statistic and the I² statistics (21).

**Association with related traits and diseases.** Secondary analyses were carried out on rs7998202, rs1046896, rs1800562, rs1799884, rs4737009, rs16926246, rs1387153, rs7998202, rs1406896, 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, 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., rs852976) acts on the mediator (e.g., FG), which in turn acts on HbA1c levels. Further details on these analyses are provided in the online 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-analyses. Association statistics with 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, MTN1RB, 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, MTN1RB, and G6PC2/ABC2B1), effect sizes were
COMMON GENETIC VARIANTS AND HbA1c

We investigated associations of HbA1c 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 HbA1c 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 HbA1c variability. The HbA1c-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 ($P < 5 \times 10^{-5}$) with erythrocyte indexes, with HbA1c-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 HbA1c levels, this time conditioning for the hematologic traits. We did not observe any difference at the three “glycemic” loci, although attenuation of $\beta$ 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. HbA1c 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, $P = 5.0 \times 10^{-5}$), 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 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 ($P = 3.61 \times 10^{-33}$) for ARIC; 5.37% (0.027) and 5.49% (0.027) ($P = 1.36 \times 10^{-5}$) 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 HbA1c 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 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 $\geq 6.5$ (%) 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>SPTA1</td>
<td>T/C</td>
<td>0.32</td>
<td>0.27</td>
<td>34,663</td>
</tr>
<tr>
<td>rs552976</td>
<td>2</td>
<td>169,616,945</td>
<td>G6PC2/ABCB11</td>
<td>G/A</td>
<td>0.66</td>
<td>0.64</td>
<td>40,420</td>
</tr>
<tr>
<td>rs1800562</td>
<td>6</td>
<td>26,201,120</td>
<td>HFE</td>
<td>G/A</td>
<td>0.96</td>
<td>0.94</td>
<td>43,778</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>45,591</td>
</tr>
<tr>
<td>rs6474359</td>
<td>8</td>
<td>1,668,351</td>
<td>ANK1</td>
<td>T/C</td>
<td>0.97</td>
<td>0.97</td>
<td>29,997</td>
</tr>
<tr>
<td>rs4737009</td>
<td>8</td>
<td>41,749,562</td>
<td>ANK1</td>
<td>A/G</td>
<td>0.28</td>
<td>0.24</td>
<td>36,862</td>
</tr>
<tr>
<td>rs16926246</td>
<td>10</td>
<td>70,763,398</td>
<td>HK1</td>
<td>C/T</td>
<td>0.89</td>
<td>0.90</td>
<td>42,707</td>
</tr>
<tr>
<td>rs1387153</td>
<td>11</td>
<td>92,313,476</td>
<td>MTNRB1</td>
<td>T/C</td>
<td>0.28</td>
<td>0.28</td>
<td>32,293</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>34,724</td>
</tr>
<tr>
<td>rs1046896</td>
<td>17</td>
<td>7,827,822</td>
<td>FN3K</td>
<td>T/C</td>
<td>0.25</td>
<td>0.31</td>
<td>45,953</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>34,562</td>
</tr>
</tbody>
</table>

*Indicates SNPs for which additional de novo genotyping was performed in eight cohorts. The

$\beta$ coefficient denotes the per-effect 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 $1.86\%$ ($p = 0.002$), 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, 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 HbA1c, 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 HbA1c. 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 HbA1c via regulation of systemic glucose concentrations for GCK, G6PC2, and 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 (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 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).

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 HK1 are associated with nonspherocytic hemolytic anemia (MIM 142600) (28,29). 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 cytoplastic 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 lower levels of HbA1c (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 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 deglycation 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 deglycation (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 preva-
## Table 3: Associations with Hba1c (%), adjusted for sex, age, and 2h-glucose, on levels of fasting or 2h-glucose

<table>
<thead>
<tr>
<th>SNP Nearest locus</th>
<th>Other</th>
<th>Effect/ (mmol/l) adjusted for 2h-glucose, sex, age</th>
<th>Effect/ (mmol/l) adjusted for Fasting glucose, sex, age</th>
<th>(SE) (N)</th>
<th>N</th>
<th>V/G</th>
<th>(SE) (N)</th>
<th>N</th>
<th>V/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs855791</td>
<td></td>
<td>GCK</td>
<td>ANK1</td>
<td>-0.024 (0.008)</td>
<td>23,654</td>
<td>0.006 (0.005)</td>
<td>23,654</td>
<td>0.020 (0.004)</td>
<td>23,508</td>
</tr>
<tr>
<td>rs1046896</td>
<td></td>
<td>G6PC2/ABCB11</td>
<td>HK1</td>
<td>-0.027 (0.006)</td>
<td>23,508</td>
<td>0.011 (0.036)</td>
<td>23,508</td>
<td>0.027 (0.004)</td>
<td>20,162</td>
</tr>
<tr>
<td>rs1387153</td>
<td></td>
<td>TMPRSS6</td>
<td>MTNR1B</td>
<td>-0.028 (0.006)</td>
<td>22,404</td>
<td>0.013 (0.009)</td>
<td>22,404</td>
<td>0.073 (0.007)</td>
<td>22,404</td>
</tr>
<tr>
<td>rs16926246</td>
<td></td>
<td>HK1</td>
<td>HK1</td>
<td>-0.023 (0.005)</td>
<td>20,162</td>
<td>0.056 (0.006)</td>
<td>20,162</td>
<td>0.027 (0.004)</td>
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<td>rs4737009</td>
<td></td>
<td>ANK1</td>
<td>ANK1</td>
<td>-0.030 (0.005)</td>
<td>23,497</td>
<td>0.018 (0.004)</td>
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<td>G6PC2/ABCB11</td>
<td>HK1</td>
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<td>23,496</td>
<td>0.097 (0.017)</td>
<td>23,496</td>
<td>0.017 (0.004)</td>
<td>23,496</td>
</tr>
</tbody>
</table>

**Hba1c** is determined from the GlucoseChi, T2D and MITZIL1 loci associations at ANK1 are shown for previous Hba1c are given for present. The SNP associations with ANK1 are shown for previous. The SNP association with ANK1 is shown for present.
It is known that conditions characterized by altered erythrocyte physiology may influence the utility of HbA1c concentrations. The apparent 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 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 top tier of HbA1c-associated common genetic variation. 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%.

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 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 HbA1c-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 HbA1c-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 HbA1c 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 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 variation 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.

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