Genetic Variant in *HK1* Is Associated With a Proanemic State and A1C but Not Other Glycemic Control–Related Traits

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OBJECTIVE—**A1C** is widely considered the gold standard for monitoring effective blood glucose levels. Recently, a genome-wide association study reported an association between **A1C** and rs7072268 within *HK1* (encoding hexokinase 1), which catalyzes the first step of glycolysis. HK1 deficiency in erythrocytes (red blood cells [RBCs]) causes severe nonspherocytic hemolytic anemia in both humans and mice.

RESEARCH DESIGN AND METHODS—The contribution of rs7072268 to **A1C** and the RBC-related traits was assessed in 6,953 nondiabetic European participants. We additionally analyzed the association with hematologic traits in 5,229 nondiabetic individuals (in whom **A1C** was not measured) and 1,924 diabetic patients. Glucose control–related markers other than **A1C** were analyzed in 18,694 nondiabetic European individuals. A type 2 diabetes case-control study included 7,447 French diabetic patients.

RESULTS—Our study confirms a strong association between the rs7072268–T allele and increased **A1C** ($\beta = 0.0299\%$; $P = 2.22 \times 10^{-7}$). Surprisingly, despite adequate study power, rs7072268 showed no association with any other markers of glucose control (fasting- and 2-h post-OGTT–related parameters, $n = 18,694$). In contrast, rs7072268–T allele decreases hemoglobin levels ($n = 13,416$; $\beta = -0.054$ g/dl; $P = 3.74 \times 10^{-9}$) and hematocrit ($n = 11,492$; $\beta = -0.13\%$; $P = 2.26 \times 10^{-4}$), suggesting a proanemic effect. The T allele also increases risk for anemia (536 cases; odds ratio 1.13; $P = 0.018$).

CONCLUSIONS—**HK1** variation, although strongly associated with **A1C**, does not seem to be involved in blood glucose control. Since **HK1** rs7072268 is associated with reduced hemoglobin levels and favors anemia, we propose that HK1 may influence A1C levels through its anemic effect or its effect on glucose metabolism in RBCs. These findings may have implications for type 2 diabetes diagnosis and clinical management because anemia is a frequent complication of the diabetes state. *Diabetes* 58:2687–2697, 2009

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**Type 2 diabetes** is a major source of early excess morbidity and mortality, which result from lack of adequate blood glucose control in most diabetic patients (1). In the absence of widely available continuous glucose monitoring, the **A1C** assay has become the most popular index to evaluate the efficiency of type 2 diabetes treatments on long-term blood glucose control (2,3). **A1C**, which is formed through the nonenzymatic attachment of glucose to the NH2-terminal of the β-chain of hemoglobin, is indeed commonly considered a surrogate marker of mean blood glucose concentration over the previous 8–12 weeks (i.e., a 120-day life span of erythrocytes) (4). Furthermore, the **A1C** assay is often used for confirming type 2 diabetes diagnosis when fasting plasma glucose (**FPG**) is in the pre-diabetes range (6.1 ≤ **FPG** < 7.0 mmol/l, defining normal glycemia and overt diabetes, respectively [2]), as postprandial or post–glucose load measurements of blood glucose are difficult to widely apply in clinical practice. However, the **A1C** measurement displays well-known caveats, such as genetically inherited hemoglobin defects or erythrocyte (red blood cell [RBC]) life span heterogeneity in hematologically normal people, that would oblige the use of more complex measurement of glycated serum proteins or fructosamine as a surrogate of blood glucose levels (5,6).

Thus far, several genome-wide association (GWA) studies have identified 22 genes or loci, increasing the risk for type 2 diabetes or modulating **FPG** levels (7–19). Recently, Pare et al. (20) reported a single nucleotide polymorphism
(SNP), rs7072268, at the hexokinase 1 (HK1) locus (chr10q22) that strongly associates with increased A1C in a nondiabetic population. The four isozymes of the hexokinase family (HK1, HK2, HK3, and glucokinase) contribute to commit glucose to the glycolytic pathway. The predominant HK1 isozyme is expressed in the vast majority of cells and tissues, including cells that are strictly dependent on glucose uptake for their metabolic needs (21). Importantly, while most tissues express more than one HK isozyme, RBC glucose metabolism only depends on HK1 activity (22). In humans, mutations including nonsynonymous substitutions in the active site of HK1 and intragenic deletions have been shown to cause HK1 enzymatic deficiency associated with autosomal recessive severe nonspherocytic hemolytic anemia (21,23–25). A similar phenotype has been described in the Downeast Anemia (dea) mice displaying HK1 deficiency (22).

Based on these observations, we postulated that HK1 genetic variation may modulate the maintenance of the RBC pool and thus indirectly alter A1C measurements independently of the ambient blood glucose concentration. We evaluated this hypothesis by assessing the impact of genetic variation through its anemic effect on HK1 activity (22). In humans, mutations including nonsynonymous substitutions in the active site of HK1 and intragenic deletions have been shown to cause HK1 enzymatic deficiency associated with autosomal recessive severe nonspherocytic hemolytic anemia (21,23–25). A similar phenotype has been described in the Downeast Anemia (dea) mice displaying HK1 deficiency (22).

**RESEARCH DESIGN AND METHODS**

**Study participants.** Clinical characteristics and data available on the studied populations are reported in Table 1. The study protocol was approved by the local ethics committee, and participants from all of the studies described (and the parents of children) signed an informed consent form.

**Genotyping of rs7072268 was performed in several cohorts**

*D.E.S.I.R.* The Data from the Epidemiological Study on the Insulin Resistance Syndrome (D.E.S.I.R.) cohort is a longitudinal French general population described elsewhere (10,26). We analyzed 4,590 nondiabetic D.E.S.I.R. participants successfully genotyped for rs7072268, of whom 3,795 were examined throughout the 9-year study.

**Swiss obese adults.** The Swiss cohort study of obese adults has previously been described (27). All of the subjects were recruited for obesity surgery. We analyzed 2,363 nondiabetic participants successfully genotyped for rs7072268.

**NFBC1986.** The Northern Finland 1986 Birth Cohort (NFBC1986) is a prospective 1-year birth cohort including all Finnish Caucasian mothers with children whose expected date of birth fell between 1 July 1985 and 30 June 1986 in the two northernmost provinces of Finland (28). Clinical examination at 15–16 years of follow-up was conducted between August 2001 and June 2002. We analyzed 5,287 nondiabetic participants successfully genotyped for rs7072268 in the NFBC1986 cohort.

**Haguenau.** The Haguenau community-based cohort of young adults investi-gates long-term consequences of being born small for gestational age and has previously been described (29). Briefly, subjects born between 1971 and 1985 were identified from a population-based registry of Haguenau (France). Non–European ancestry subjects are estimated to be <0.1% of the general population (29). At a mean age of 22 years, participants under overnight fasting conditions underwent a medical examination for assessment of anthropometric and clinical parameters. We analyzed 1,455 unaffected nondiabetic participants successfully genotyped for rs7072268.

**Obesity French pedi-grees.** French children and adults with European ancestry from families with a history of obesity were recruited at the Centre National de la Recherche Scientifique (CNRS)-UMB8900 unit (Lille, France) through an ongoing national media campaign (30). We analyzed 5,261 nondiabetic participants successfully genotyped for rs7072268.

**French type 2 diabetes case-control study.** We analyzed 7,447 unrelated French individuals with type 2 diabetes ascertainment from the French type 2 diabetes family and Obesity family studies, collected by the CNRS-UMB8900 unit, from the Endocrinology-Diabetology Department of the Corbeil-Essonnes Hospital (7), and from the Diab harassment (Diab2-Néphrogéné/Surdiagène

**TABLE 1 Clinical characteristics and data available on the study populations with successful genotyping for rs7072268**

| Study populations | D.E.S.I.R. at baseline | NFBC1986 | Haguenau | French obese adults | French children from obesity pedigrees | French adults from obesity pedigrees | French control subjects |
|-------------------|------------------------|-----------|----------|---------------------|----------------------------------------|--------------------------------------|-------------------------|
| n (male/female)   | 4,590 (2,259/2,331)    | 2,363     | 5,287    | 2,363               | 5,287                                  | 2,363                                | 5,287                   |
| BMI (kg/m²)       | 24.6 ± 5.0             | 24.6 ± 5.0| 24.6 ± 5.0| 24.6 ± 5.0          | 24.6 ± 5.0                             | 24.6 ± 5.0                         | 24.6 ± 5.0             |
| Age (years)       | 42.1 ± 10.0            | 42.1 ± 10.0| 42.1 ± 10.0| 42.1 ± 10.0         | 42.1 ± 10.0                            | 42.1 ± 10.0                        | 42.1 ± 10.0            |
| Fasting glucose (mmol/l) | 5.14 ± 0.76            | 5.14 ± 0.76| 5.14 ± 0.76| 5.14 ± 0.76         | 5.14 ± 0.76                            | 5.14 ± 0.76                       | 5.14 ± 0.76            |
| Fasting insulin (pmol/l) | 48.7 ± 20.9            | 48.7 ± 20.9| 48.7 ± 20.9| 48.7 ± 20.9         | 48.7 ± 20.9                            | 48.7 ± 20.9                      | 48.7 ± 20.9            |
| A1C (%)           | 5.59 ± 0.48            | 5.59 ± 0.48| 5.59 ± 0.48| 5.59 ± 0.48         | 5.59 ± 0.48                            | 5.59 ± 0.48                       | 5.59 ± 0.48            |
| **Association study with rs7072268** | | | | | | | |
study (31). We used 5,380 unrelated normoglycemic participants (age at exam ≥40 years) as control subjects (ascertained by the D.E.S.I.R. cohort; the SU.Vi.MAX study, which has previously been described [32], and the French type 2 diabetes family and obesity family studies). For each population, glycemic status was defined according to 1997 American Diabetes Association criteria (2): normal glucose was defined as FPG <6.1 mmol/l without hypoglycemic treatment, and type 2 diabetes was defined as FPG ≥7.0 mmol/l or treatment with antidiabetic agents. For the Corbeil study, overt nephropathy was defined as microalbuminuria levels ≥30 mg/24 h or ≥20 mg/l in two of three urinary takings.

**Genotyping.** Genotyping of SNP rs7072268 was performed using a TaqMan assay according to the manufacturer’s instructions (no. C-300059210; Applied Biosystems, Foster City, CA). Allelic discrimination was performed by capillary electrophoresis analysis using an Applied Biosystems 3730xl DNA Analyzer and GeneMapper 3.7 software. The genotype success rate was at least 98%, and no deviation (P > 0.05) from Hardy-Weinberg equilibrium was observed in any of the examined populations. The genotyping of MTNR1B-rs18830963, GCK-rs1579084, G6PC2-rs5608887, and SLC30A8-rs1266634 in the D.E.S.I.R. study had previously been reported (10,19,33,34).

**Statistical analyses.** We analyzed the effect of SNP rs7072268 on quantitative traits using linear regression models under an additive model adjusted for age, sex, and BMI. To take into account familial relationships within the French obesity pedigrees, we tested the association between rs7072268 and glucose homeostasis-related traits using Gaussian models of generalized estimated equations (GEEs) performed with STATA software. The estimates of the effect of rs7072268 on quantitative traits and their standard errors for each separate population were combined in the meta-analyses using the weighted inverse normal method. The overall effect and its CI were estimated using the inverse variance method implemented in the “meta.summaries” function of the R RMETA package. The effect of rs7072268 on diabetic status was assessed using a logistic regression model adjusted for age, sex, and BMI. In the D.E.S.I.R. participants, the effect of the rs7072268 genotype on quantitative traits was assessed in nondiabetic individuals at baseline and using repeated measures at 3-, 6-, and 9-year follow-up visits. We used mixed models for analyses of repeated measures adjusted for age, sex, and BMI. Using the QUANTO software, we estimated what significant effects of rs7072268 on quantitative traits was assessed in nondiabetic individuals at baseline and using repeated measures at 3, 6, and 9-year follow-ups visits. We used mixed models for analyses of repeated measures adjusted for age, sex, and BMI. Using the QUANTO software, we estimated what significant effects of rs7072268 on quantitative traits and their standard errors for each separate population were combined in the meta-analyses using the weighted inverse normal method. The overall effect and its CI were estimated using the inverse variance method implemented in the “meta.summaries” function of the R RMETA package. The effect of rs7072268 on diabetic status was assessed using a logistic regression model adjusted for age, sex, and BMI. In the D.E.S.I.R. participants, the effect of the rs7072268 genotype on quantitative traits was assessed in nondiabetic individuals at baseline and using repeated measures at 3, 6, and 9-year follow-up visits. We used mixed models for analyses of repeated measures adjusted for age, sex, and BMI. Using the QUANTO software, we estimated what significant effects of rs7072268 on glucose homeostasis-related parameters can be detected with a power of 80%. All statistical analyses were performed with R (version 2.6.1), SPSS (version 14.0 for Windows), QUANTO (version 1.2), and STATA software (version 5.0).

**Indexes calculation.** Homeostasis model assessment of pancreatic β-cell function (HOMA-B) was calculated as follows: HOMA-B = (20 * fasting serum insulin/FPG) – 3.5, where fasting serum insulin is in milliunits per liter and FPG is in millimoles per liter (35). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = (FPG * fasting serum insulin)/22.5, where fasting serum insulin is in picomoles per liter and FPG is in millimoles per liter (35).

The insulinogenic index, the insulin sensitivity index (ISI), and the disposition index (DI) were calculated from an oral glucose tolerance test (OGTT) according to the following formulas:

Insulinogenic index = (serum insulin at 30 min – fasting serum insulin)/plasma glucose at 30 min, where serum insulin is in picomoles per liter and plasma glucose is in millimoles (36).

\[ ISI = \frac{10,000}{\sqrt{\text{FPG} \times \text{fasting serum insulin}} \times \text{mean OGTTglucose}} \times \text{mean OGTT_{f0-120}} \], where serum insulin is in milliunits per liter and plasma glucose is in millimoles per liter (36).

\[ DI = ISI \times 100 \times \text{serum insulin at 30 min} / \text{plasma glucose at 30 min} \times (\text{plasma glucose at 30 min} - 3.85) \], where serum insulin is in milliunits per liter and plasma glucose is in milliunits per liter (38).

**RESULTS**

**SNP rs7072268 strongly associates with increased A1C level in nondiabetic individuals.** We first genotyped SNP rs7072268 in 4,590 middle-aged nondiabetic...
individuals from the French D.E.S.I.R. population (mean age 47 years) and in 2,363 Swiss nondiabetic obese adults (mean age 41 years) (Table 1). After an additive genetic model adjusted for age, sex, and BMI was applied, the SNP rs7072268 does not associate with any other markers of glucose control in nondiabetic individuals.

We then assessed the impact of the rs7072268–T allele on glucose homeostasis–related traits in the D.E.S.I.R. and Swiss samples. After applying an additive genetic model adjusted for age, sex, and BMI, we did not find significant associations between rs7072268 and any glucose-related traits including fasting glucose, fasting insulin, HOMA-B, and HOMA-IR (Table 3).

To further support these paradoxical findings, we tested the effect of rs7072268 on the same fasting traits in 12,003 additional nondiabetic individuals ascertained from the NFBC1986 study (age at examination 16 years), the French Haguenau cohort (mean age 22 years), and French obesity pedigrees including both children and adults (mean age 11 and 46 years, respectively) (Table 1). A1C levels were not measured in these sample sets. After applying an identically adjusted additive genetic model, we did not find
significant associations with any of these traits as analyzed in each cohort or in the overall combined meta-analysis (Table 3). Furthermore, analyses of glucose and insulin levels after an oral glucose load in 1,440 individuals from Haguenau and in 1,055 children and 2,294 adults from the French obesity pedigrees did not show any significant associations (Table 4).

**SNP rs7072268 associates with RBC-related parameters and anemia in nondiabetic individuals.** Since our results thus far suggested that the effect of rs7072268 on A1C was not due to differences in glycemic status, we assessed the impact of rs7072268 on RBC-related parameters available in D.E.S.I.R. and the Swiss obese adults sample set and also in 5,229 participants from the NFBC1986 study (where RBC-related traits but not A1C were measured). After an additive genetic model adjusted for age, sex, and BMI was applied, our combined analysis demonstrated an association between the rs7072268 –T allele and decreased hematocrit (Table 5) and decreased hemoglobin levels (Table 5). Combined case-control studies for anemia (stringently defined by hemoglobin ≤12 g/dl for women and ≤13 g/dl for men; 669 cases) from the same cohorts further supported the anemic effect of the rs7072268–T allele (odds ratio [OR] 1.13 [95% CI 1.01–1.27]; combined analysis).
| T-allele frequency | n     | RBC-related parameters | Mean data level by genotype (SD unless otherwise indicated) | Per T-allele effect (9% CI)* | P     |
|------------------|-------|------------------------|-----------------------------------------------------------|-----------------------------|-------|
|                  |       |                        | CC       | CT       | TT       |                                     |       |
| D.E.S.I.R. at baseline | 0.49  | 4,576                  | RBC count (×10^{12}/L) | 4.82 ± 0.41 | 4.79 ± 0.41 | 4.78 ± 0.41 | -0.018 (-0.025 to -0.011) | 8.01 × 10^{-3} |
|                  |       |                        | Hematocrit (%) | 43.60 ± 3.61 | 43.50 ± 3.61 | 43.28 ± 3.67 | -0.18 (-0.24 to -0.12) | 2.11 × 10^{-3} |
|                  |       |                        | Hemoglobin (g/dl) | 14.41 ± 1.26 | 14.36 ± 1.24 | 14.30 ± 1.28 | -0.064 (-0.074 to -0.035) | 5.20 × 10^{-3} |
|                  |       |                        | MCH (pg/cell) | 29.95 ± 1.54 | 30.00 ± 1.57 | 29.94 ± 1.64 | 0.98                     |       |
|                  |       |                        | MCV (×10^{-15} /cell) | 90.73 ± 4.18 | 90.88 ± 4.33 | 90.65 ± 4.34 | 0.68                     |       |
|                  |       |                        | MCHC (%)     | 33.01 ± 0.96 | 33.01 ± 1.06 | 33.03 ± 0.97 | 0.57                     |       |
| Swiss obese adults | 0.54  | 1,687                  | RBC count (×10^{12}/L) | 4.81 ± 0.37 | 4.84 ± 0.39 | 4.84 ± 0.38 | 0.31                     |       |
|                  |       |                        | Hematocrit (%) | 43.19 ± 3.32 | 43.19 ± 3.36 | 42.93 ± 3.10 | -0.17 (-0.27 to -0.070) | 0.087 |
|                  |       |                        | Hemoglobin (g/dl) | 14.35 ± 1.19 | 14.28 ± 1.26 | 14.22 ± 1.24 | -0.081 (-0.115 to -0.046) | 0.019 |
|                  |       |                        | MCH (pg/cell) | 29.86 ± 1.77 | 29.68 ± 1.85 | 29.46 ± 2.22 | -0.21 (-0.28 to -0.14) | 2.16 × 10^{-3} |
|                  |       |                        | MCV (×10^{-15} /cell) | 90.05 ± 4.77 | 89.55 ± 4.56 | 88.92 ± 5.39 | -0.56 (-0.72 to -0.38) | 1.29 × 10^{-3} |
|                  |       |                        | MCHC (%)     | 33.16 ± 1.18 | 33.15 ± 1.12 | 33.11 ± 1.18 | 0.29                     |       |
| NFBC1986         | 0.40  | 5,229                  | RBC count (×10^{12}/L) | 4.71 ± 0.40 | 4.70 ± 0.42 | 4.70 ± 0.42 | 0.66                     |       |
|                  |       |                        | Hematocrit (%) | 40.67 ± 3.35 | 40.49 ± 3.53 | 40.49 ± 3.55 | -0.086 (-0.137 to -0.035) | 0.094 |
|                  |       |                        | Hemoglobin (g/dl) | 13.77 ± 1.20 | 13.71 ± 1.23 | 13.20 ± 1.28 | -0.030 (-0.047 to -0.012) | 0.087 |
|                  |       |                        | MCH (pg/cell) | 29.40 ± 1.77 | 29.31 ± 1.87 | 29.30 ± 1.85 | 0.12                     |       |
|                  |       |                        | MCV (×10^{-15} /cell) | 86.42 ± 4.05 | 86.28 ± 4.21 | 86.32 ± 4.45 | 0.45                     |       |
|                  |       |                        | MCHC (%)     | 33.89 ± 0.95 | 33.84 ± 0.98 | 33.86 ± 0.97 | 0.24                     |       |
| Meta-analysis    | —     | 11,492                 | RBC count (×10^{12}/L) | — | — | — | -0.0068 (-0.015 to 0.0015) | 0.11 |
|                  |       |                        | Hematocrit (%) | — | — | — | -0.13 (-0.20 to -0.06) | 2.26 × 10^{-4} |
|                  |       |                        | Hemoglobin (g/dl) | — | — | — | -0.044 (-0.071 to -0.017) | 1.43 × 10^{-3} |
|                  |       |                        | MCH (pg/cell) | — | — | — | NA†                     |       |
|                  |       |                        | MCV (×10^{-15} /cell) | — | — | — | NA†                     |       |
|                  |       |                        | MCHC (%)     | — | — | — | 0.0005 (-0.036 to 0.037) | 0.42 |
| Corbeil type 2 diabetes study | 0.52  | 1,924                  | Hemoglobin (g/dl) | 14.30 ± 1.32 | 14.25 ± 1.33 | 14.07 ± 1.35 | -0.13 (-0.16 to -0.09) | 7.66 × 10^{-4} |
|                  |       |                        | MCV (×10^{-15} /cell) | 90.26 ± 6.20 | 90.07 ± 5.49 | 89.63 ± 6.10 | -0.03 (-0.051 to -0.15) | 0.070 |
| Overall meta-analysis   | —     | 13,416                 | Hemoglobin (g/dl) | — | — | — | -0.064 (-0.076 to -0.031) | 3.74 × 10^{-6} |
|                  |       |                        | MCV (×10^{-15} /cell) | — | — | — | NA†                     |       |
| D.E.S.I.R. over the 9-year follow-up study‡ | 0.49  | 15,119                 | RBC count (×10^{12}/L) | — | — | — | -0.020 (-0.027 to -0.014) | 9.63 × 10^{-4} |
|                  |       |                        | Hematocrit (%) | — | — | — | -0.17 (-0.22 to -0.12) | 3.73 × 10^{-4} |
|                  |       |                        | Hemoglobin (g/dl) | — | — | — | -0.065 (-0.071 to -0.038) | 1.04 × 10^{-3} |
|                  |       |                        | MCH (pg/cell) | — | — | — | 0.43                      |       |
|                  |       |                        | MCV (×10^{-15} /cell) | — | — | — | 0.72                      |       |
|                  |       |                        | MCHC (%)     | — | — | — | 0.55                      |       |

Data are means ± SD unless otherwise indicated. Associations between rs7072268 and RBC-related parameters were assessed applying an additive model adjusted for age, sex, and BMI. *Per T-allele effect size: the regression coefficient β. The T-allele effect is only displayed for when P < 0.10. †P < 0.05 for heterogeneity in effects on both MCH and MCV indices. We thus considered these two traits not applicable for overall meta-analyses. ‡P values and regression coefficients β are calculated from mixed additive models. MCHC, MCV concentration; NA, not applicable.
TABLE 6
French type 2 diabetes case-control analyses according to SNP rs7072268

| T-allele frequency | n    | CC          | CT          | TT          | OR (95% CI)* | P      |
|-------------------|------|-------------|-------------|-------------|-------------|--------|
| Type 2 diabetic participants | 0.51 | 7,447       | 1,784 (0.24) | 3,708 (0.50) | 1,955 (0.26) | Ref.   |
| Control subjects  | 0.50 | 5,380       | 1,327 (0.25) | 2,715 (0.50) | 1,338 (0.25) | 1.069 (1.001–1.142) | 0.045 |

Data are n (frequency) unless otherwise indicated. Type 2 diabetes was defined according to 1997 American Diabetes Association criteria (2).

*OR from additive logistic regression models adjusted for age, sex, and BMI.

$P = 0.032$). We next studied the effects of variation at rs7072268 on mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) indexes: because the $P$ values for heterogeneity in effects on both traits were <0.05, our analysis was performed in each cohort in isolation. In Swiss obese adults, the rs7072268–T allele associates with both decreased MCH and MCV parameters ($\beta = -0.21 \text{ pg/cell} [95\% \text{ CI} -0.28 \text{ to } -0.14], P = 2.16 \times 10^{-3}$, and $\beta = -0.56 \times 10^{-15} \text{ l/cell} [-0.72 \text{ to } -0.38], P = 1.29 \times 10^{-3}$, respectively; Table 5), suggesting a microspherocytic anemic state. In the D.E.S.I.R. participants, the RBC count also showed a negative association with the rs7072268–T allele both at baseline ($\beta = -0.018 \times 10^{12}/\text{l} [95\% \text{ CI} -0.025 \text{ to } -0.011], P = 8.01 \times 10^{-3}$, Table 5) and over the 9-year follow-up ($\beta = -0.020 \times 10^{12}/\text{l} [-0.027 \text{ to } -0.014], P = 9.63 \times 10^{-3}$, respectively; Table 5).

**Effect of SNP rs7072268 on RBC-related parameters in type 2 diabetic individuals.** The rs7072268–T allele was also associated with decreased hemoglobin level in 1,924 French type 2 diabetic subjects from the Corbeil Hospital cohort, in whom this parameter was measured ($\beta = -0.13 \text{ g/dl} [95\% \text{ CI} -0.16 \text{ to } -0.09], P = 7.66 \times 10^{-4}$, Table 5). When the presence of overt nephropathy, the microalbuminuria level, or the albumin-to-creatinine ratio were introduced in the linear regression model, this association remained significant ($P < 1.5 \times 10^{-3}$), suggesting that the effect of HK1 on RBC is independent of diabetes-linked kidney disease. We also identified in type 2 diabetic subjects a trend for association between the rs7072268–T allele and decreased MCV (Table 5).

**Combined meta-analysis of SNP rs7072268 on RBC-related parameters.** In a combined meta-analysis including nondiabetic and type 2 diabetic participants, the rs7072268–T allele strongly associated with decreased hemoglobin levels ($n = 13,416$; $\beta = -0.054 \text{ g/dl} [95\% \text{ CI} -0.076 \text{ to } -0.031]$, combined $P = 3.74 \times 10^{-6}$, Table 5). In addition, the trend for an increased risk for clinical anemia was further supported (836 cases; OR 1.13 [95% CI 1.02–1.25]; combined $P = 0.018$).

**Impact of SNP rs7072268 on type 2 diabetes risk.** We then assessed the contribution of rs7072268 to type 2 diabetes risk in 7,447 French type 2 diabetic individuals and 5,380 unrelated normoglycemic French control subjects (age at exam $\geq$40 years). The type 2 diabetes case-control analysis only displayed a nominal association between the rs7072268–T allele and increased risk of type 2 diabetes (OR 1.07 [95% CI 1.00–1.14], $P = 0.045$; Table 6). These findings were not supported by GWA studies meta-analyses carried out by the DIAGRAM+ consortium, including 8,130 type 2 diabetic and 38,987 control European participants (OR 0.98 [0.94–1.02]; $P = 0.40$) (M. McCarthy, unpublished data). Therefore, the weak HK1 rs7072268 effect on increased type 2 diabetes risk, found in our samples, is not supported by other European populations.

**Impact of the five established genetic determinants of A1C on A1C levels, FPG, and RBC-related parameters in D.E.S.I.R.** We then analyzed the contribution of four previously reported genetic determinants of A1C (MTNR1B-rs10830963 [9,34], GCK-rs1799884 [20], G6PC2-rs560887 [20], and SLC30A8-rs13266634 [20]) on A1C levels in the D.E.S.I.R. cohort. We confirmed the contribution of these SNPs to A1C levels in $\sim$4,500 nondiabetic individuals from the D.E.S.I.R. study at baseline—except for SLC30A8-rs13266634, which displayed only a trend for association with A1C levels ($P_{MTNR1B} = 2.25 \times 10^{-4}$, $P_{GCK} = 1.32 \times 10^{-1}$, $P_{G6PC2} = 2.31 \times 10^{-6}$, and $P_{SLC30A8} = 0.063$; Table 7). Analysis of HK1-rs7072263 combined with the four other SNPs demonstrated a significant additive effect on A1C levels ($\beta_{per \text{ allele}} = 0.032\%$, $P = 1.49 \times 10^{-15}$, Fig. 1). Individuals carrying seven or more “high-A1C” alleles ($n = 415$; $\sim$11% of the European population) showed a mean 0.17% increase in A1C compared with individuals carrying fewer than two high-A1C alleles ($n = 219$; Fig. 1).

We then examined the effect of MTNR1B-rs10830963, GCK-rs1799884, G6PC2-rs560887, and SLC30A8-rs13266634 on FPG levels and RBC-related parameters including RBC count, hemoglobin, and hematocrit levels. As previously reported (9,10,19,33), the four SNPs are strongly associated with FPG levels (Table 7). SNPs GCK-rs1799884, G6PC2-rs560887, and SLC30A8-rs13266634 are not associated with RBC-related parameters (Table 7). In contrast, the MTNR1B-rs10830963–T allele associates with decreased RBC count and hemoglobin and hematocrit levels ($\beta = -0.017 \times 10^{-2}/\text{l} [95\% \text{ CI} -0.025 \text{ to } -0.001], P = 0.022$; $\beta = -0.055 \text{ g/dl} [-0.076 \text{ to } -0.033], P = 0.011$; and $\beta = -0.19% \text{ hematocrit} [-0.25 \text{ to } -0.12], P = 4.13 \times 10^{-3}$, respectively; Table 7).

**DISCUSSION**

Our data unambiguously demonstrate that HK1 rs7072268 strongly associates with increased A1C levels in European general populations, as reported by Pare et al. (20). In contrast, we failed to find any further association with other quantitative metabolic traits commonly used to monitor glucose control. In addition, it is unlikely that HK1 rs7072268 significantly increases risk for type 2 diabetes. Our data suggest that the effect of HK1 variation on A1C levels may be due to a molecular mechanism involving RBC function rather than related to impaired blood glucose homeostasis. In this regard, we found that the HK1 rs7072268–T allele increasing A1C is strongly associated with reduced hemoglobin and hematocrit levels (Spearman correlation between hematocrit and hemoglobin levels in nondiabetic subjects from D.E.S.I.R.: $r^2 = 0.94$; $P < 0.0001$). In addition, the rs7072268–T allele contributes to an increase in the risk of clinical anemia. However, this result has to be confirmed in large-scale and more powered case-control studies. In support of our
findings, dea mice with an HK1 deficiency also display lower RBC count and hemoglobin and hematocrit levels (22). Indeed, these mice show severe anemia, with extensive tissue iron deposition and marked reticulocytosis, which results from significant intravascular hemolysis (22). Approximately 20 patients with nonspherocytic hemolytic anemia due to HK1 deficiency have been described thus far (21), but there is no information available about their A1C levels. SNP rs7072268 is located in the first intron of the HK1 isoform, HK1-R, specifically expressed in RBC and is in intermediate linkage disequilibrium with a common nonsynonymous coding SNP, rs1133189 (according to the HapMap CEU population: \( r^2 = 0.58 \)). Although we have no obvious information about the truly causative common SNPs in the HK1 locus associated with anemia (that might be obtained from fine-mapping studies), we speculate they may impair HK1 expression or the maturation of this hexokinase enzymatic isoform in reticulocytes and in mature RBCs, as known in monogenic HK1 deficiency (21,23).

In RBCs, the oxygen affinity of hemoglobin is strongly regulated by 2,3-biphosphoglycerate (2,3-DPG) produced by a bypass in glycolysis (21). Increasing 2,3-DPG levels cause a decreased oxygen affinity and thus improve the

### Table 7

| SNP          | A1C (%)      | Fasting glucose (mmol/l) | Hemoglobin (g/dl) | Hematocrit (%) | RBC count (\( \times 10^{12}/l \)) |
|--------------|--------------|--------------------------|-------------------|----------------|-----------------------------------|
| HK1 rs7072268-T | 0.023 (0.016–0.031) | -0.004 (–0.014 to 0.006) | -0.054 (–0.074 to –0.035) | -0.18 (–0.24 to –0.12) | -0.018 (–0.025 to –0.011) |
| MTNR1B rs10830963-G | 0.031 (0.023–0.039) | 0.093 (0.082–0.104) | 5.20 \( \times 10^{-3} \) | 2.11 \( \times 10^{-3} \) | 8.01 \( \times 10^{-3} \) |

Associations between SNPs and quantitative traits were assessed with the application of an additive model adjusted for age, sex, and BMI.

**FIG. 1.** Cumulative effect of HK1-rs7072268, MTNR1B-rs10830963, GCK-rs1799884, G6PC2-rs560887, and SLC30A8-rs13266634 on A1C in nondiabetic individuals of the D.E.S.I.R. study. A linear regression model was carried out with application of an additive model adjusted for age, sex, and BMI. Data are presented as means [95% CI]. The β-coefficient corresponds with the increase in A1C levels (%) by additional high-A1C alleles. The numbers of individuals per category of high-A1C alleles and corresponding percentages are shown below the graph.
transfer of oxygen to tissues and ameliorate the anemic state. HK1 deficiency contributes to decrease 2,3-DPG levels and thus annuls its beneficial effect (21). HK1 is also known to bind in mitochondria to the voltage-dependent anion channels, known as mitochondrial porins (39). Mitochondrial-associated hexokinase activity has been shown to protect cells from entering apoptosis via the blockade of the interaction of the proapoptotic BAX with mitochondrial-associated hexokinase activity has been shown to protect cells from entering apoptosis via the blockade of the interaction of the proapoptotic BAX with other genetic determinants of A1C (9). Other studies are needed for confirmation of these findings. A1C was affected by adjustment for the hemoglobin or hematocrit levels (supplemental Table A1, available in the online appendix, available at http://diabetes.journals.org/cgi/content/full/db09-0652/DC1). This may suggest that the hemoglobin or hematocrit levels (frequency: 0.30; 95% CI 0.058 to 0.13) modulate fasting glucose but do not influence glucose or hematologic parameters. As both the American Diabetes Association and the European Association for the Study of Diabetes have proposed to use A1C as a criterion for type 2 diabetes diagnosis (an individual with A1C <6% is considered as nondiabetic), both genetic and environmental factors (including iron and vitamin B12) interacting with RBC function and survival have to be taken into consideration to better interpret A1C levels in the general population. Furthermore, diabetes by itself is a known cause for anemia through a range of deleterious mechanisms (44), and it would be important to better determine the impact of anemia on A1C assays.

In conclusion, our study presents mechanisms that may underlie the consistent association between HK1 genetic variation and A1C but also identifies for the first time a gene contributing to a common proanemic state. At a time when the utility of GWA studies is debated for disease prediction (50), our study highlights the power of GWA to identify physiological determinants of complex conditions such as anemia having serious implications for health.

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TABLE 7
Continued

| GCK rs1799884-A (frequency: 0.27; n = 4,406) | G6PC2 rs560887-A (frequency: 0.30; n = 4,339) | SLC30A8 rs13266634-T (frequency: 0.30; n = 4,488) |
|-------------------------------------------|---------------------------------|----------------------------------|
| **β (95% CI)**                            | **β (95% CI)**                   | **β (95% CI)**                   |
| 0.038 (0.028 to 0.048)                    | 0.132 × 10⁻⁴                    | 0.016 (0.024 to 0.007)           |
| 0.054 (0.041 to 0.067)                    | 0.63 × 10⁻⁸                     | 0.039 (0.050 to 0.028)           |
| 0.023 (0.002 to 0.049)                    | 0.010 (0.002 to 0.031)          | 0.65 (0.017 to 0.026)            |
| 0.020 (0.058 to 0.097)                    | 0.066 (0.009 to 0.13)           | 0.008 (0.057 to 0.073)           |
| 0.001 (0.008 to 0.010)                    | 0.0005 (0.007 to 0.008)         | 0.002 (0.005 to 0.010)           |

The mechanism by which HK1-related anemia increases A1C levels is unknown. Using a conditional regression model, we failed to clearly show that the HK1 effect on A1C was affected by adjustment for the hemoglobin or hematocrit levels (supplemental Table A1, available in the online appendix, available at http://diabetes.journals.org/cgi/content/full/db09-0652/DC1). This may suggest that the hemoglobin or hematocrit levels would explain a small variance of A1C. However, larger studies are needed for confirmation of these findings. A higher turnover of the RBC pool should diminish protein glycation as a result of the reduced hemoglobin half-life (5). Alternatively, we speculate that the enhanced accumulation of unprocessed glucose resulting from the HK1 deficiency may favor hemoglobin glycation within RBCs, which in turn may increase the RBC death rate via their impaired deformability (44). Importantly, anemia due to iron deficiency often seen in late pregnancy also causes increased A1C levels (45), and A1C levels significantly increase after iron or vitamin B12 treatment in patients with iron or vitamin B12 deficiency anemia, respectively (46,47). Therefore, different anemia-inducing mechanisms increase A1C levels.

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with a history of obesity and type 2 diabetes. We are grateful to the DIAGRAM+ consortium for providing GWA studies data on rs7072268, and we particularly thank Mark McCarthy and Benjamin Voight. The Diab-2-Néphroge`ne/ Surdia`gène study acknowledges the participating patients and physicians and the staff of the CIC Poitiers, PHRC (Projet Hospitalier de Recherche Clinique). We thank Leena Petlonen for providing NFBC1986 DNA samples.

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