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 European 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 ($\beta = 0.029\%$; $P = 2.22 \times 10^{-7}$). 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; $P = 3.74 \times 10^{-6}$) 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 (836 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**

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 NH_2-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 ($6.1 \leq$ 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

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See accompanying commentary, p. 2444.

(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 *HK1* rs7072268 on A1C, other glucose control-related traits, type 2 diabetes risk, and RBC-related parameters in several prospective and case-control European cohorts. Our data suggest that *HK1* variation through its anemic effect impairs A1C assays, which may have important clinical implications for both type 2 diabetes diagnosis and management because anemia is commonly associated with diabetes.

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 investigates 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 nondiabetic participants successfully genotyped for rs7072268.

Obesity French pedigrees. 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)-UMR8090 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 ascertained from the French type 2 diabetes family and Obesity family studies, collected by the CNRS-UMR8090 unit, from the Endocrinology-Diabetology Department of the Corbeil-Essonnes Hospital (7), and from the Diabhycar/Diab2-Néphrogène/Surdiagène

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

Study populations	D.E.S.I.R. at baseline	Swiss obese adults	NFBC1986	Haguenau	French children from obesity pedigrees	French adults from obesity pedigrees	Type 2 diabetes case-control study: French type 2 diabetic case subjects	Type 2 diabetes case-control study: French control subjects
n (male/female)	4,590 (2,259/2,331)	2,363 (511/1,852)	5,287 (2,628/2,659)	1,455 (690/765)	1,411 (678/733)	3,850 (1,454/2,396)	7,447 (4,752/2,695)	5,380 (2,293/3,087)
Age (years)	47.1 ± 10.0	40.8 ± 11.1	16.0	22.1 ± 3.9	11.4 ± 3.3	46.3 ± 15.2	62.7 ± 10.3	53.0 ± 8.3
BMI (kg/m²)	24.6 ± 3.7	43.1 ± 7.2	21.3 ± 3.7	22.6 ± 4.1	26.2 ± 7.4	32.5 ± 9.4	30.7 ± 6.2	25.2 ± 5.0
Fasting glucose (mmol/l)	5.3 ± 0.5	5.1 ± 0.6	5.2 ± 0.4	4.8 ± 0.4	4.9 ± 0.5	5.3 ± 0.7	NA	NA
Fasting insulin (pmol/l)	39.2 (28.6–55.8)	110.4 (75.9–165.6)	66.2 (51.2–85.6)	32.3 (22.2–43.8)	69.0 (42.8–109.7)	55.9 (33.3–89.7)	NA	NA
A1C (%)	5.43 ± 0.40	5.59 ± 0.48	NA	NA	NA	NA	NA	NA
Association study with rs7072268	■	■	■	■	■	■		
A1C	■	■	■	■	■	■		
Fasting metabolic traits	■	■	■	■	■	■		
Metabolic traits during an OGTT								
RBC-related parameters	■	■	■				■*	

Data are means ± SD or medians (interquartile range). *RBC-related parameters were only available in type 2 diabetic patients from the Corbeil-Hospital. ■, Available data for the association study with rs7072268. NA, not applicable or not available.

TABLE 2
Association of rs7072268 with A1C level in nondiabetic individuals from the D.E.S.I.R. study (at baseline and over the 9-year follow-up study) and from the Swiss obese adults sample set

	n	T-allele frequency	Mean A1C level by genotype (% A1C)			Additive model adjusted for age, sex, and BMI		Additive model adjusted for age, sex, BMI, and FPG level	
			CC	CT	TT	Per T-allele effect: A1C*	*P*	Per T-allele effect: A1C*	*P*
D.E.S.I.R. at baseline	4,590	0.49	5.40 ± 0.41	5.43 ± 0.39	5.45 ± 0.39	0.023 (0.016–0.031)	1.76×10^{-3}	0.026 (0.018–0.033)	4.03×10^{-4}
Swiss obese adults	2,363	0.54	5.56 ± 0.45	5.58 ± 0.48	5.64 ± 0.49	0.046 (0.032–0.060)	9.46×10^{-4}	0.035 (0.026–0.044)	1.13×10^{-4}
Meta-analysis	6,953	—				0.028 (0.016–0.041)	1.53×10^{-5}	0.029 (0.018–0.040)	2.22×10^{-7}
D.E.S.I.R. over the 9-year follow-up study†	15,073	0.49				0.022 (0.016–0.029)	3.93×10^{-4}	0.023 (0.017–0.029)	1.20×10^{-4}

Data are means ± SD or percent (95% CI) unless otherwise indicated. Association between rs7072268 and A1C was assessed applying an additive model adjusted for age, sex, and BMI or adjusted for age, sex, BMI, and FPG. †*P* values and regression coefficients β are calculated from mixed models described in the STATISTICAL ANALYSES section. *Per T-allele effect size: the regression coefficient β.

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-30005592-10; Applied Biosystems, Foster City, CA). Allelic discrimination was performed by capillary electrophoresis analysis using an Applied Biosystems 3730xl DNA Analyser 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. Genotyping of *MTNR1B*-rs10830963, *GCK*-rs1799884, *G6PC2*-rs560887, and *SLC30A8*-rs13266634 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 glucose homeostasis–related parameters we could expect in the related meta-analyses, with a detection power of 80%. Given the analyzed sample sizes, small effects of *HK1* rs7072268 (estimated at $\beta \leq 0.1$) 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).

$$\text{ISI} = 10{,}000/\sqrt{(\text{FPG} \times \text{fasting serum insulin} \times \text{mean OGTT}_{\text{glucose}} \times \text{mean OGTT}_{\text{insulin}})}$$

where serum insulin is in milliunits per liter and plasma glucose is in millimoles per liter (37).

$$\text{DI} = \text{ISI} \times 100 \times \text{serum insulin at 30 min}[\text{plasma glucose at 30 min} \times (\text{plasma glucose at 30 min} - 3.89)]$$

where serum insulin is in milliunits per liter and plasma glucose is in millimoles per liter (38).

ISI and DI were only calculated in the French obese pedigrees as measurements of serum insulin and plasma glucose were available at 0, 30, 60, 90, and 120 min after glucose load. In the Haguenau study, measurements of serum insulin and plasma glucose were only available at 0, 30, and 120 min after glucose load.

RESULTS

SNP rs7072268 strongly associates with increased A1C level in nondiabetic individuals. We first genotyped SNP rs7072268 in 4,590 middle-aged nondiabetic

TABLE 3
Associations between rs7072268 and glucose homeostasis–related traits in nondiabetic individuals from several European cohorts

Glucose homeostasis–related traits	T-allele frequency	*n*	Mean data level by genotype			*P*
			CC	CT	TT	
D.E.S.I.R.	0.49	4,590				
Fasting glucose (mmol/l)			5.29 ± 0.53	5.27 ± 0.52	5.28 ± 0.54	0.66
Fasting insulin (pmol/l)			39.22 (28.63–56.82)	39.15 (28.54–55.61)	39.58 (28.82–55.78)	0.78
HOMA-B			67.65 (48.06–94.05)	67.67 (49.19–95.41)	69.51 (49.52–93.61)	0.79
HOMA-IR			9.15 (6.43–13.66)	9.17 (6.48–13.23)	9.19 (6.44–13.72)	0.74
Swiss obese adults	0.54	2,101				
Fasting glucose (mmol/l)			5.14 ± 0.63	5.11 ± 0.58	5.16 ± 0.57	0.44
Fasting insulin (pmol/l)			103.5 (69–158.7)	110.4 (75.9–165.6)	110.4 (75.9–172.2)	0.08
HOMA-B			200.0 (132.5–306.1)	216.0 (137.7–329.2)	200.0 (137.5–314.3)	0.24
HOMA-IR			24.2 (15.5–36.2)	24.9 (16.9–36.7)	25.5 (16.3–38.3)	0.10
NFBC1986	0.40	5,287				
Fasting glucose (mmol/l)			5.15 ± 0.44	5.15 ± 0.43	5.14 ± 0.41	0.81
Fasting insulin (pmol/l)			66.24 (51.06–87.63)	66.24 (51.06–84.67)	67.62 (51.06–86.25)	0.53
HOMA-B			118.67 (90.00–156.67)	117.89 (92.00–156.21)	120.00 (90.00–156.67)	0.75
HOMA-IR			15.03 (11.50–20.16)	15.12 (11.43–19.77)	15.35 (11.57–19.73)	0.52
Haguenau	0.52	1,455				
Fasting glucose (mmol/l)			4.76 ± 0.35	4.80 ± 0.38	4.79 ± 0.39	0.29
Fasting insulin (pmol/l)			33.01 (22.96–44.49)	33.01 (22.96–44.49)	30.49 (21.53–43.59)	0.82
HOMA-B			78.09 (50.61–112.93)	75.09 (51.23–107.05)	72.82 (50.06–104.93)	0.74
HOMA-IR			7.08 (4.79–9.48)	6.97 (4.89–9.55)	6.57 (4.48–9.34)	0.72
French children from obesity pedigrees	0.49	1,411				
Fasting glucose (mmol/l)			4.89 ± 0.47	4.93 ± 0.48	4.86 ± 0.51	0.30
Fasting insulin (pmol/l)			68.31 (42.78–107.30)	68.31 (42.44–107.30)	70.38 (44.85–112.47)	0.89
HOMA-B			151.76 (99.44–225.38)	145.33 (94.87–229.43)	152.73 (94.44–253.33)	0.66
HOMA-IR			15.13 (8.89–23.09)	14.95 (8.98–24.04)	14.72 (9.44–25.07)	0.98
French adults from obesity pedigrees	0.51	3,850				
Fasting glucose (mmol/l)			5.33 ± 0.68	5.34 ± 0.69	5.36 ± 0.67	0.76
Fasting insulin (pmol/l)			54.17 (33.12–84.70)	55.20 (33.12–89.01)	58.65 (34.50–93.84)	0.25
HOMA-B			87.55 (56.32–141.14)	93.52 (57.38–142.71)	96.36 (59.64–151.54)	0.45
HOMA-IR			12.74 (7.42–20.62)	13.26 (7.43–21.42)	14.03 (7.82–23.30)	0.23
Overall meta-analysis	—	18,694				
Fasting glucose (mmol/l)						0.93
Fasting insulin (pmol/l)						0.79
HOMA-B						0.90
HOMA-IR						0.81

Data are means ± SD or, for logarithmically transformed data, medians (interquartile range). Associations between rs7072268 and glucose homeostasis–related traits were assessed applying an additive model adjusted for age, sex, and BMI—except for the NFBC1986 (an adjustment for sex and BMI was only performed because all of the subjects were 16 years old). Data for fasting serum insulin, HOMA-B, and HOMA-IR were logarithmically transformed before statistical analysis.

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 rs7072268–T allele showed a consistent association with increased A1C in the D.E.S.I.R. study at baseline and over the 9-year follow-up ($\beta = 0.023\%_{A1C}$ [95% CI 0.016–0.031], $P = 1.76 \times 10^{-3}$, and $\beta = 0.022\%_{A1C}$ [0.016–0.029], $P = 3.93 \times 10^{-4}$, respectively; Table 2) and in the Swiss obese adults sample set ($\beta = 0.046\%_{A1C}$ [0.032–0.060], $P = 9.46 \times 10^{-4}$; Table 2). These results were unchanged when the additive genetic model was adjusted for age and sex only (data not shown). When we also included FPG level in the linear regression model, the significance of the effect on A1C was stronger in both studies and in a meta-analysis of the D.E.S.I.R. baseline data and the Swiss obese samples ($n = 6{,}953$; $\beta = 0.029\%_{A1C}$ [0.018–0.040], combined $P = 2.22 \times 10^{-7}$; Table 2).

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

TABLE 4
Associations between rs7072268 and quantitative metabolic traits during an OGTT in nondiabetic French individuals from the Haguenau study and obesity pedigrees

Quantitative metabolic traits during an OGTT	Data level by genotype			*P*
	CC	CT	TT	
French children from obesity pedigrees with T-allele frequency 0.49 (n = 1,055)				
Plasma glucose (mmol/l)				
30-min post-OGTT	7.24 ± 1.42	7.20 ± 1.52	7.29 ± 1.49	0.85
120-min post-OGTT	5.47 ± 1.13	5.39 ± 1.18	5.39 ± 1.16	0.22
Serum insulin*				
30-min post-OGTT	498 (283–732)	448 (275–698)	461 (274–763)	0.57
120-min post-OGTT	206 (107–411)	193 (99–401)	213 (100–451)	0.72
Insulinogenic index*	58.7 (34.5–84.7)	54.4 (31.6–82.4)	54.3 (33.4–89.9)	0.96
ISI*	32.5 (21.3–55.4)	37.0 (23.4–58.1)	33.8 (21.3–57.2)	0.43
DI*	10,025 (5,539–18,125)	10,827 (6,013–18,391)	9,012 (5,345–16,832)	0.83
French children from obesity pedigrees with T-allele frequency 0.51 (n = 2,294)				
Plasma glucose (mmol/l)				
30-min post-OGTT	8.22 ± 1.67	8.40 ± 1.90	8.32 ± 1.85	0.70
120-min post-OGTT	5.68 ± 1.95	5.72 ± 1.92	5.78 ± 1.97	0.43
Serum insulin*				
30-min post-OGTT	293 (167–490)	305 (182–481)	295 (165–485)	0.97
120-min post-OGTT	168 (79–366)	182 (83–370)	190 (91–364)	0.27
ISI*	106.6 (60.2–192.6)	102.4 (62.3–170.0)	107.0 (57.5–174.8)	0.46
DI*	13,046 (6,496–26,909)	12,806 (6,130–25,563)	13,005 (5,841–24,931)	0.41
Haguenau with T-allele frequency 0.52 (n = 1,440)				
Plasma glucose (mmol/l)				
30-min post-OGTT	7.51 ± 1.42	7.61 ± 1.46	7.49 ± 1.40	0.60
120-min post-OGTT	5.40 ± 1.22	5.30 ± 1.14	5.27 ± 1.18	0.17
Serum insulin*				
30-min post-OGTT	294 (185–445)	287 (187–420)	287 (181–434)	0.82
120-min post-OGTT	165 (93–266)	172 (108–273)	165 (101–266)	0.99
Insulinogenic index*	34.9 (20.6–53.6)	33.1 (21.6–50.8)	34.8 (21.6–50.9)	0.87
Overall meta-analysis (n = 4,789)				
Plasma glucose (mmol/l)				
30-min post-OGTT				0.99
120-min post-OGTT				0.24
Serum insulin*				
30-min post-OGTT				0.71
120-min post-OGTT				0.83
Insulinogenic index*				0.84
ISI*				0.92
DI*				0.42

Data are means ± SD or, for logarithmically transformed data, median (interquartile range). Associations between rs7072268 and quantitative metabolic traits during an OGTT were assessed applying an additive model adjusted for age, sex, and BMI. Meta-analyses of both ISI and DI included association data of the participants from French obesity pedigrees only. *Data logarithmically transformed before statistical analysis.

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 (n = 11,492; $\beta = -0.13\%_{\text{hematocrit}}$ [95% CI −0.20 to −0.06], combined $P = 2.26 \times 10^{-4}$; Table 5) and decreased hemoglobin levels ($\beta = -0.044$ g/dl [−0.071 to −0.017], combined $P = 1.43 \times 10^{-3}$; 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

TABLE 5
Associations between rs7072268 and RBC-related parameters in nondiabetic individuals from D.E.S.I.R. (at baseline and over the 9-year follow-up), the Swiss obese adults, and the NFBC1986 and in diabetic French participants from the Corbeil type 2 diabetes study

	T-allele frequency	*n*	RBC-related parameters	Mean data level by genotype			Per T-allele effect (95% CI)*	*P*
				CC	CT	TT		
D.E.S.I.R. at baseline	0.49	4,576	RBC count ($\times 10^{12}$/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 (%)	43.66 ± 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	−0.054 (−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 ($\times 10^{-15}$ l/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 ($\times 10^{12}$/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 ($\times 10^{-15}$ l/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 ($\times 10^{12}$/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 ($\times 10^{-15}$ l/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 ($\times 10^{12}$/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†	NA†
			MCV ($\times 10^{-15}$ l/cell)				NA†	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 ($\times 10^{-15}$ l/cell)	90.26 ± 6.20	90.07 ± 5.49	89.63 ± 6.10	−0.33 (−0.51 to −0.15)	0.070
Overall meta-analysis	—	13,416	Hemoglobin (g/dl)				−0.054 (−0.076 to −0.031)	3.74×10^{-6}
			MCV ($\times 10^{-15}$ l/cell)				NA†	NA†
D.E.S.I.R. over the 9-year follow-up study‡	0.49	15,119	RBC count ($\times 10^{12}$/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)				−0.055 (−0.071 to −0.038)	1.04×10^{-3}
			MCH (pg/cell)					0.43
			MCV ($\times 10^{-15}$ l/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, MCH 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 heterogenity 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 ($\beta = -0.21$ pg/cell [95% CI −0.28 to −0.14], $P = 2.16 \times 10^{-3}$, and $\beta = -0.56 \times 10^{-15}$ l/cell [−0.72 to −0.38], $P = 1.29 \times 10^{-3}$, respectively; Table 5), suggesting a microspherocytocytic 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 ($\beta = -0.018 \times 10^{12}$/l [95% CI −0.025 to −0.011], $P = 8.01 \times 10^{-3}$; Table 5) and over the 9-year follow-up ($\beta = -0.020 \times 10^{12}$/l [−0.027 to −0.014], $P = 9.63 \times 10^{-4}$, 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 ($\beta = -0.13$ g/dl [95% CI −0.16 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 ($n = 13{,}416$; $\beta = -0.054$ g/dl [95% CI −0.076 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 ≥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 ~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 ($P_{MTNR1B} = 2.25 \times 10^{-4}$, $P_{GCK} = 1.32 \times 10^{-4}$, $P_{G6PC2} = 2.31 \times 10^{-6}$, 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 ($\beta_{\text{per allele}} = 0.032\%$, $P = 1.49 \times 10^{-15}$; Fig. 1). Individuals carrying seven or more "high-A1C" alleles ($n = 415$; ~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 assessed 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 ($\beta = -0.017 \times 10^{12}$/l [95% CI −0.025 to −0.001], $P = 0.022$; $\beta = -0.055$ g/dl [−0.076 to −0.033], $P = 0.011$; and $\beta = -0.19\%$ hematocrit [−0.25 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

TABLE 7
Association of A1C, fasting glucose, hemoglobin, hematocrit, and RBC count with candidate SNPs in nondiabetic participants of the D.E.S.I.R. study at baseline

	HK1 rs7072268-T (frequency: 0.49; n = 4,590)		*MTNR1B* rs10830963-G (frequency: 0.28; n = 4,597)	
	β (95% CI)	*P*	β (95% CI)	*P*
A1C (%)	0.023 (0.016–0.031)	1.76×10^{-3}	0.031 (0.023–0.039)	2.25×10^{-4}
Fasting glucose (mmol/l)	−0.004 (−0.014 to 0.006)	0.66	0.093 (0.082–0.104)	1.32×10^{-16}
Hemoglobin (g/dl)	−0.054 (−0.074 to −0.035)	5.20×10^{-3}	−0.055 (−0.076 to −0.033)	0.011
Hematocrit (%)	−0.18 (−0.24 to −0.12)	2.11×10^{-3}	−0.19 (−0.25 to −0.12)	4.13×10^{-3}
RBC count ($\times 10^{12}$/l)	−0.018 (−0.025 to −0.011)	8.01×10^{-3}	−0.017 (−0.025 to −0.0097)	0.022

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

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

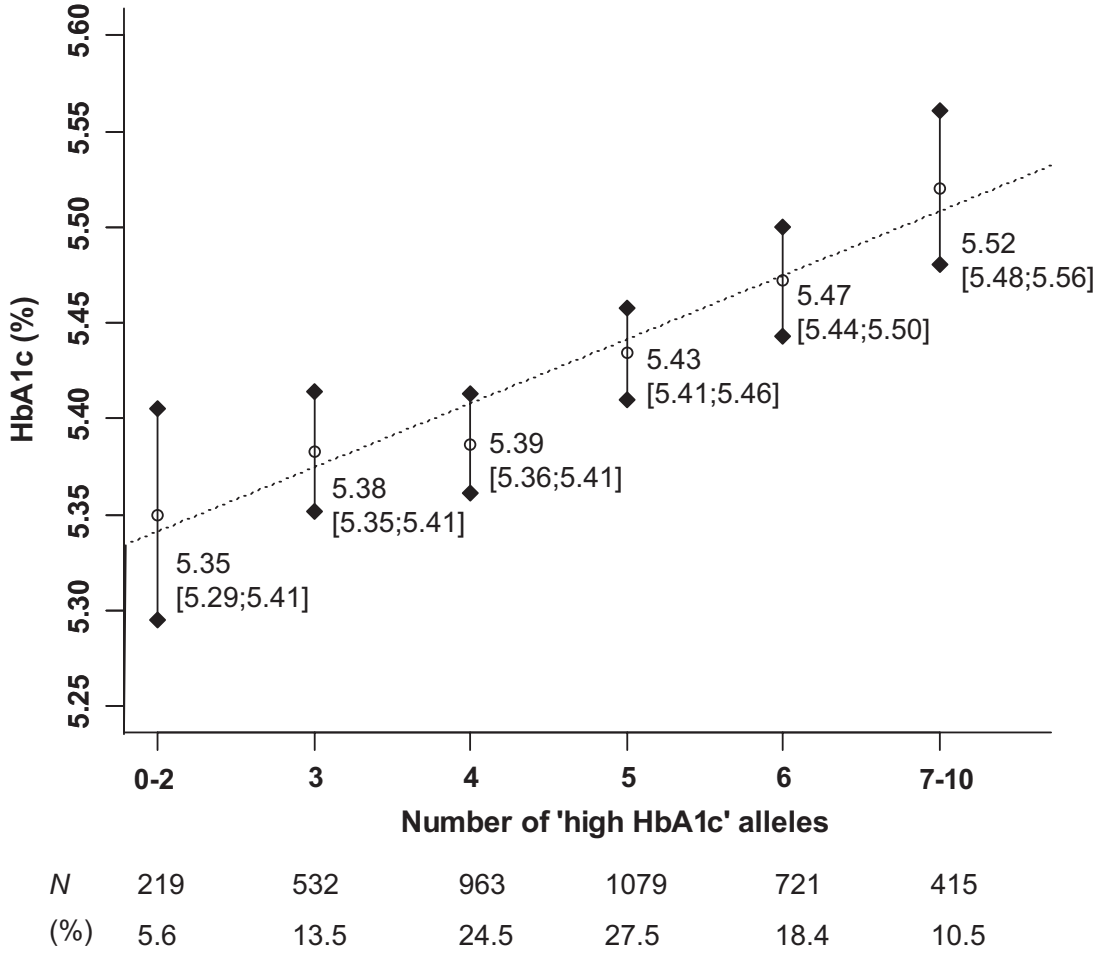

FIG. 1. Cumulative effect of *HK1*-rs7072268, *MTNR1B*-rs10830963, *GCK*-rs1799884, *G6PC2*-rs560887, and *SLC30A8*-rs13266634 on A1C in nondiabetic individuals from the D.E.S.I.R. study. A linear regression model was carried out with application of an additive model adjusted for age, sex, and BMI. Data 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.

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)	P	β (95% CI)	P	β (95% CI)	P
0.038 (0.028 to 0.048)	1.32×10^{-4}	−0.040 (−0.049 to −0.032)	2.31×10^{-6}	−0.016 (−0.024 to −0.007)	0.063
0.054 (0.041 to 0.067)	4.63×10^{-5}	−0.077 (−0.089 to −0.066)	4.72×10^{-12}	−0.039 (−0.050 to −0.028)	4.54×10^{-4}
0.023 (−0.002 to 0.049)	0.35	0.010 (−0.012 to 0.031)	0.65	0.004 (−0.017 to 0.026)	0.85
0.020 (−0.058 to 0.097)	0.80	0.066 (0.0009 to 0.13)	0.31	0.008 (−0.057 to 0.073)	0.91
0.001 (−0.008 to 0.010)	0.88	0.0005 (−0.007 to 0.008)	0.94	0.002 (−0.005 to 0.010)	0.78

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 the voltage-dependent anion channels (40–42). We speculate that *HK1* variation may impair the HK1 antiapoptotic effect in reticulocytes (i.e., the precursors of RBCs), as well as in kidney and brain where *HK1* is expressed (21,43). It may have deleterious effects on maturation of RBCs and on erythropoiesis via decreased synthesis of kidney and brain erythropoietin (Epo).

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.diabetesjournals.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 decrease 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.

Other genes associated with RBC-related parameters may also interfere with the glycation of hemoglobin. In this regard, our present data suggest that genetic variation in *MTNR1B* (encoding melatonin receptor 2), which strongly influences both A1C and fasting glucose (9), also associates with decreased RBC count and hemoglobin and hematocrit levels. Melatonin is a neurohormone mainly involved in the regulation of circadian rhythms. Recently, Bozek et al. (48) provided evidence of a circadian oscillation of *Epo* gene expression in the kidney, a tissue that strongly expresses *MTNR1B* in rats (49). In contrast, three other genetic determinants of A1C (*GCK*, *G6PC2*, and *SLC30A8*) modulate fasting glucose but do not influence hematologic parameters measured in our cohorts. Altogether, A1C levels seem to be largely genetically determined (Fig. 1), possibly via the modulation of blood 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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REFERENCES

1. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560–2572
2. American Diabetes Association: Standards of medical care in diabetes (Position Statement). Diabetes Care 2008;31(Suppl. 1):S12–S54
3. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984;310:341–346
4. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50:2239–2244
5. Cohen RM, Smith EP. Frequency of HbA1c discordance in estimating blood glucose control. Curr Opin Clin Nutr Metab Care 2008;11:512–517
6. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, Palascak MB, Joiner CH. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 2008;112:4284–4291
7. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881–885
8. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PI, Abecasis GR, Almgren P, Andersen G, Ardlie K, Bostrom KB, Bergman RN, Bonnycastle LL, Borch-Johnsen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren WL, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hitman GA, Hughes TE, Isomaa B, Jackson AU, Jorgensen T, Kong A, Kubalanza K, Kuruvilla FG, Kuusisto J, Langenberg C, Lango H, Lauritzen T, Li Y, Lindgren CM, Lyssenko V, Marvelle AF, Meisinger C, Midthjell K, Mohlke KL, Morken MA, Morris AD, Narisu N, Nilsson P, Owen KR, Palmer CN, Payne F, Perry JR, Pettersen E, Platou C, Prokopenko I, Qi L, Qin L, Rayner NW, Rees M, Roix JJ, Sandbaek A, Shields B, Sjogren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ, Illig T, Hveem K, Hu FB, Laakso M, Stefansson K, Pedersen O, Wareham NJ, Barroso I, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638–645
9. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, Sparso T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, De Graeve F, Chevre JC, Borch-Johnsen K, Hartikainen AL, Ruokonen A, Tichet J, Marre M, Weill J, Heude B, Tauber M, Lemaire K, Schuit F, Elliott P, Jorgensen T, Charpentier G, Hadjadj S, Cauchi S, Vaxillaire M, Sladek R, Visvikis-Siest S, Balkau B, Levy-Marchal C, Pattou F, Meyre D, Blakemore AI, Jarvelin MR, Walley AJ, Hansen T, Dina C, Pedersen O, Froguel P. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet 2009;41:89–94
10. Bouatia-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, Cavalcanti-Proenca C, Marchand M, Hartikainen AL, Sovio U, De Graeve F, Rung J, Vaxillaire M, Tichet J, Marre M, Balkau B, Weill J, Elliott P, Jarvelin MR, Meyre D, Polychronakos C, Dina C, Sladek R, Froguel P. A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. Science 2008;320:1085–1088
11. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–1345
12. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, McCarthy MI, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–1341
13. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331–1336
14. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007;39:770–775
15. Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orru M, Grazia Piras M, Bonnycastle LL, Willer CJ, Lyssenko V, Shen H, Kuusisto J, Ebrahim S, Sestu N, Duren WL, Spada MC, Stringham HM, Scott LJ, Olla N, Swift AJ, Najjar S, Mitchell BD, Lawlor DA, Smith GD, Ben-Shlomo Y, Andersen G, Borch-Johnsen K, Jorgensen T, Saramies J, Valle TT, Buchanan TA, Shuldiner AR, Lakatta E, Bergman RN, Uda M, Tuomilehto J, Pedersen O, Cao A, Groop L, Mohlke KL, Laakso M, Schlessinger D, Collins FS, Altshuler D, Abecasis GR, Boehnke M, Scuteri A, Watanabe RM. Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Invest 2008;118:2620–2628
16. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orru M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR. Variants in MTNR1B influence fasting glucose levels. Nat Genet 2009;41:77–81
17. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spegel P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M, Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 2009;41:82–88
18. Sparso T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T, Pedersen O. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia 2008;51:70–75
19. Vaxillaire M, Veslot J, Dina C, Proenca C, Cauchi S, Charpentier G, Tichet J, Fumeron F, Marre M, Meyre D, Balkau B, Froguel P. Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. Diabetes 2008;57:244–254
20. Pare G, Chasman DI, Parker AN, Nathan DM, Miletich JP, Zee RY, Ridker

PM. Novel association of HK1 with glycated hemoglobin in a non-diabetic population: a genome-wide evaluation of 14,618 participants in the Women's Genome Health Study. PLoS Genet 2008;4:e1000312
21. van Wijk R, van Solinge WW. The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis. Blood 2005;106:4034–4042
22. Peters LL, Lane PW, Andersen SG, Gwynn B, Barker JE, Beutler E. Downeast anemia (dea), a new mouse model of severe nonspherocytic hemolytic anemia caused by hexokinase (HK(1)) deficiency. Blood Cells Mol Dis 2001;27:850–860
23. van Wijk R, Rijksen G, Huizinga EG, Nieuwenhuis HK, van Solinge WW. HK Utrecht: missense mutation in the active site of human hexokinase associated with hexokinase deficiency and severe nonspherocytic hemolytic anemia. Blood 2003;101:345–347
24. McMullin MF. The molecular basis of disorders of red cell enzymes. J Clin Pathol 1999;52:241–244
25. Kanno H, Murakami K, Hariyama Y, Ishikawa K, Miwa S, Fujii H. Homozygous intragenic deletion of type I hexokinase gene causes lethal hemolytic anemia of the affected fetus. Blood 2002;100:1930
26. Balkau B. An epidemiologic survey from a network of French Health Examination Centres (D.E.S.I.R.): epidemiologic data on the insulin resistance syndrome. Rev Epidemiol Sante Publique 1996;44:373–375 [in French]
27. Steffen R, Potoczna N, Bieri N, Horber FF. Successful multi-intervention treatment of severe obesity: a 7-year prospective study with 96% follow-up. Obes Surg 2009;19:3–12
28. Jarvelin MR, Elliott P, Kleinschmidt I, Martuzzi M, Grundy C, Hartikainen AL, Rantakallio P. Ecological and individual predictors of birthweight in a northern Finland birth cohort 1986. Paediatr Perinat Epidemiol 1997;11:298–312
29. Jaquet D, Collin D, Levy-Marchal C, Czernichow P. Adult height distribution in subjects born small for gestational age. Horm Res 2004;62:92–96
30. Meyre D, Lecoeur C, Delplanque J, Francke S, Vatin V, Durand E, Weill J, Dina C, Froguel P. A genome-wide scan for childhood obesity-associated traits in French families shows significant linkage on chromosome 6q22.31–q23.2. Diabetes 2004;53:803–811
31. Hadjadj S, Fumeron F, Roussel R, Saulnier PJ, Gallois Y, Ankotche A, Travert F, Abi Khalil C, Miot A, Alhenc-Gelas F, Lievre M, Marre M. Prognostic value of the insertion/deletion polymorphism of the ACE gene in type 2 diabetic subjects: results from the Non-insulin-dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR), Diabete de type 2, Nephropathie et Genetique (DIAB2NEPHROGENE), and Survie, Diabete de type 2 et Genetique (SURDIAGENE) studies. Diabetes Care 2008;31:1847–1852
32. Hercberg S, Preziosi P, Briancon S, Galan P, Triol I, Malvy D, Roussel AM, Favier A. A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population: the SU.VI.MAX study: design, methods, and participant characteristics. SUpplementation en VItamines et Mineraux AntioXydants. Control Clin Trials 1998;19:336–351
33. Cauchi S, Proenca C, Choquet H, Gaget S, De Graeve F, Marre M, Balkau B, Tichet J, Meyre D, Vaxillaire M, Froguel P. Analysis of novel risk loci for type 2 diabetes in a general French population: the D.E.S.I.R. study. J Mol Med 2008;86:341–348
34. Sparso T, Bonnefond A, Andersson E, Bouatia-Naji N, Holmkvist J, Wegner L, Grarup N, Gjesing AP, Banasik K, Cavalcanti-Proenca C, Marchand M, Vaxillaire M, Charpentier G, Jarvelin MR, Tichet J, Balkau B, Marre M, Levy-Marcha C, Faerch K, Borch-Johnsen K, Jorgensen T, Madsbad S, Poulsen P, Vaag A, Dina C, Hansen T, Pedersen O, Froguel P: The G-allele of intronic rs10830963 in MTNR1B confers increased risk of impaired fasting glycemia and type 2 diabetes through an impaired glucose-stimulated insulin release: studies involving 19,605 Europeans. Diabetes 2009;58:1450–1456
35. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–1495
36. Wareham NJ, Phillips DI, Byrne CD, Hales CN. The 30 minute insulin incremental response in an oral glucose tolerance test as a measure of insulin secretion. Diabet Med 1995;12:931
37. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–1470
38. Bergman RN, Ader M, Huecking K, Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. Diabetes 2002;51(Suppl. 1):S212–S220
39. da-Silva WS, Gomez-Puyou A, de Gomez-Puyou MT, Moreno-Sanchez R, De Felice FG, de Meis L, Oliveira MF, Galina A. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. J Biol Chem 2004;279:39846–39855
40. Pastorino JG, Shulga N, Hoek JB. Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis. J Biol Chem 2002;277:7610–7618
41. Bryson JM, Coy PE, Gottlob K, Hay N, Robey RB. Increased hexokinase activity, of either ectopic or endogenous origin, protects renal epithelial cells against acute oxidant-induced cell death. J Biol Chem 2002;277:11392–11400
42. Arzoine L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V. Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. J Biol Chem 2009;284:3946–3955
43. Gardiner NJ, Wang Z, Luke C, Gott A, Price SA, Fernyhough P. Expression of hexokinase isoforms in the dorsal root ganglion of the adult rat and effect of experimental diabetes. Brain Res 2007;1175:143–154
44. Singh DK, Winocour P, Farrington K. Erythropoietic stress and anemia in diabetes mellitus. Nat Rev Endocrinol 2009;5:204–210
45. Hashimoto K, Noguchi S, Morimoto Y, Hamada S, Wasada K, Imai S, Murata Y, Kasayama S, Koga M. A1C but not serum glycated albumin is elevated in late pregnancy owing to iron deficiency. Diabetes Care 2008;31:1945–1948
46. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 2004;112:126–128
47. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med 1990;227:133–136
48. Bozek K, Relogio A, Kielbasa SM, Heine M, Dame C, Kramer A, Herzel H. Regulation of clock-controlled genes in mammals. PLoS ONE 2009;4:e4882
49. Ishii H, Tanaka N, Kobayashi M, Kato M, Sakuma Y. Gene structures, biochemical characterization and distribution of rat melatonin receptors. J Physiol Sci 2009;59:37–47
50. Hirschhorn JN. Genomewide association studies: illuminating biologic pathways. N Engl J Med 2009;360:1699–1701