

A Genome-Wide Association Study Identifies a Novel Major Locus for Glycemic Control in Type 1 Diabetes, as Measured by Both A1C and Glucose

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OBJECTIVE—Glycemia is a major risk factor for the development of long-term complications in type 1 diabetes; however, no specific genetic loci have been identified for glycemic control in individuals with type 1 diabetes. To identify such loci in type 1 diabetes, we analyzed longitudinal repeated measures of A1C from the Diabetes Control and Complications Trial.

RESEARCH DESIGN AND METHODS—We performed a genome-wide association study using the mean of quarterly A1C values measured over 6.5 years, separately in the conventional ($n = 667$) and intensive ($n = 637$) treatment groups of the DCCT. At loci of interest, linear mixed models were used to take advantage of all the repeated measures. We then assessed the association of these loci with capillary glucose and repeated measures of multiple complications of diabetes.

RESULTS—We identified a major locus for A1C levels in the conventional treatment group near *SORCS1* (10q25.1, $P = 7 \times 10^{-10}$), which was also associated with mean glucose ($P = 2 \times 10^{-5}$). This was confirmed using A1C in the intensive treatment group ($P = 0.01$). Other loci achieved evidence close to genome-wide significance: 14q32.13 (*GSC*) and 9p22 (*BNC2*) in the combined treatment groups and 15q21.3 (*WDR72*) in the inten-

sive group. Further, these loci gave evidence for association with diabetic complications, specifically *SORCS1* with hypoglycemia and *BNC2* with renal and retinal complications. We replicated the *SORCS1* association in Genetics of Diabetes in Kidneys (GoKinD) study control subjects ($P = 0.01$) and the *BNC2* association with A1C in nondiabetic individuals.

CONCLUSIONS—A major locus for A1C and glucose in individuals with diabetes is near *SORCS1*. This may influence the design and analysis of genetic studies attempting to identify risk factors for long-term diabetic complications. *Diabetes* 59:539–549, 2010

Elevation in plasma glucose, as measured by A1C, is a major risk factor for long-term diabetic complications (1–13). Twin and family studies have shown that A1C levels are heritable in nondiabetic individuals (14–16). In addition, significant correlation in A1C between monozygotic twins both concordant and discordant for type 1 diabetes (14,17), as well as in siblings with type 1 diabetes (18,55), suggests that some genetic factors influence A1C in both diabetic and nondiabetic individuals. However, no study of dizygous twins has been performed. These observations have motivated genome-wide linkage studies of measures of glycemia (typically single fasting plasma glucose or A1C), predominantly in nondiabetic individuals, but have not led to the identification of novel genes possibly because of small effect sizes of individual loci and underpowered studies (15,19,20). More recently, association studies of fasting glucose in nondiabetic individuals have identified multiple loci that meet genome-wide significance criteria ($P < 5 \times 10^{-8}$, supplementary Table 1, available in an online appendix at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-0653/DC1>) (20–29).

In individuals with type 1 diabetes, there is considerable variability in glycemic control both between and within individuals. Intra-individual variation is likely caused by changes in diabetes management (e.g., pump versus multiple daily injections [30]), diet, activity, weight control, and insulin dosing in response to glucose and A1C measures. Other factors that may influence A1C are erythrocyte turnover, hemoglobinopathies, defects of glucose transport into erythrocytes, mechanisms of glycation and de-glycation, and alterations in intracellular glucose metabolism. Specifically, in the conventional treatment arm of the Diabetes Control and Complications Trial (DCCT),

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Received 1 May 2009 and accepted 20 October 2009. Published ahead of print at <http://diabetes.diabetesjournals.org> on 20 October 2009. DOI: 10.2337/db09-0653. Clinical trial registry nos. NCT00360815 (DCCT) and NCT00360893 (EDIC), clinicaltrials.gov.

*A complete list of investigators of MAGIC is provided in online appendix supplementary Table 10, available at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-0653/DC1>.

†A complete list of investigators and members of the research group appears in *N Engl J Med* 2005;353:2643–2653.

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See accompanying commentary, p. 332.

TABLE 1
Descriptive information of 1,304 white DCCT subjects, separately by treatment group

	Conventional	Intensive
Sex (n)		
Male	363	332
Female	304	305
Cohort (n)		
Primary prevention	344	307
Secondary intervention	323	330
Age at DCCT baseline (years)	26.5 ± 7.1	27.2 ± 7.1
Duration in the DCCT (years)	6.2 ± 1.6	6.3 ± 1.7
Eligibility A1C (%)	9.00 ± 1.61	9.07 ± 1.58
A1C measures obtained*	26 ± 7 (5–40)	63 ± 21 (3–103)
Mean A1C (%)*	9.06 ± 1.24	7.22 ± 0.93
Mean daily glucose from seven-point capillary profile (mg/dl)*	231 ± 80	156 ± 50
Stimulated C-peptide at DCCT baseline (pmol/ml)	0.117 ± 0.119	0.111 ± 0.119

Data are means ±SD and means ±SD (range) unless otherwise indicated. There were 667 individuals in the conventional group and 637 in the intensive group with genotype data. *For the intensive group, values used were from DCCT year 1 onward, whereas in the conventional group, all DCCT values were used, and for A1C in the conventional group, quarterly values were used, while in the intensive group, monthly values were used.

the intra-class correlation between consecutive quarterly A1C values was 0.79, falling to 0.42 for values measured 3 years apart. In the face of such high variability over time, analyzing longitudinally repeated measures via mixed models can increase power to identify risk factors. Alternatively, taking the mean of the values over time may be sufficient to initially screen for important genetic effects. We hypothesized that common genetic variants associated with glycemic control in subjects with type 1 diabetes could be identified using repeated measures of A1C from subjects in the DCCT. Because glycemic control was the major intervention in DCCT, we performed genetic analysis separately in each treatment group, with the expectation that genetic effects may be larger in the conventional treatment group in which there was no attempt to alter A1C (apart from preventing very high values [i.e., >13.11%]).

RESEARCH DESIGN AND METHODS

DCCT subjects. A total of 1,441 patients with type 1 diabetes were recruited for the DCCT (1,311), which was designed to determine if intensive diabetes management, with the goal of normalizing glycemic levels, would prevent or delay the development and/or progression of long-term diabetic complications. At screening for eligibility, A1C had to be >6.6%, and subjects with hemoglobinopathies were excluded (31). Subjects were randomized to either conventional therapy (CON) or intensive therapy (INT) (Table 1). CON consisted of one or two daily injections of mixed intermediate and short-acting insulins, daily self-monitoring of urine initially or later blood glucose, and education about diet and exercise (31,32), but did not usually include daily adjustments in the insulin dosage. INT included the administration of insulin three or more times daily by injection or treatment with an external pump. Insulin doses were adjusted according to the results of self-monitoring of blood glucose performed four or more times per day, dietary intake, and anticipated exercise. The goals of INT included preprandial blood glucose 70–120 mg/dl, postprandial <180 mg/dl, a weekly 3:00 A.M. measurement >65 mg/dl, and monthly A1C within the normal range (<6.05%). Women who became pregnant or were planning a pregnancy received INT until the time of delivery. The DCCT was terminated prematurely in 1993, when it was conclusively shown that intensive management delayed the development and progression of retinopathy, nephropathy, and neuropathy (1). DNA was collected (33), and participants gave written informed consent for its use.

These studies were approved by the institutional review boards of all participating institutions.

A1C, glucose, and C-peptide measurements. DCCT subjects had A1C measured centrally using a high-performance liquid chromatography method once during eligibility screening and quarterly (CON) or monthly (INT) during DCCT, for between 3 and 9 years (mean 6.5 years, Table 1) (34). The stability of this assay over time has been described (34). The mean quarterly A1C level in each CON participant during DCCT was 9.06% (SD 1.24), based on an average of 26 measures (Table 1, supplementary Fig. 1). The mean monthly A1C measures in INT from DCCT year 1 onward was 7.22% (SD 0.96, supplementary Fig. 7). A centrally measured capillary blood glucose daily profile was obtained quarterly, consisting of seven samples, obtained before and 90 min after each meal, and one before bedtime, from which a daily mean was calculated. Stimulated C-peptide was measured at screening for DCCT eligibility (35). CON subjects were masked to their centrally measured A1C, glucose, and C-peptide results (31). Further, at 82% of their visits, they were not adjusting their insulin dose in response to self-monitoring of blood glucose (30).

Genotyping and quality control. We performed genome-wide genotyping in DCCT subjects using the Illumina 1M beadchip assay (www.illumina.com, San Diego, CA). Genotypes were called using BeadStudio using all individuals at once. Data from three probands was excluded because of discrepancies between reported sex and genotype data. No data were removed because of low genotype call rate (minimum call rate threshold was 0.988). A total of 58 probands were removed because of disagreements between genotypes of single-nucleotide polymorphisms (SNPs) from an earlier study (33). Genotypes from 24 duplicate samples had an agreement rate of 99.9995%. Sample contamination was assessed by calculation of the mean heterozygosity across the genome for each individual, and using a range of 0.25–0.32, none were removed. Consistency of genotypes with Mendelian inheritance was observed in 28 trios with an observed rate of 99.71%. To detect cryptically related individuals and/or sample mix-ups, identical-by-state estimates between all pairs of individuals were performed (36), and two probands were removed. A total of 841,342 SNPs with a minor allele frequency >1% were subsequently analyzed statistically.

Autosomal SNPs showing significant association with sex ($P < 10^{-8}$) or deviating from the Hardy-Weinberg equilibrium ($P < 10^{-8}$) were excluded from the analysis. To reduce the possibility of population stratification, we limited the analysis to individuals who self-identified as white and excluded individuals who were determined to be admixed between Caucasian and other ethnic groups through population genetic approaches (37), seeding with genotype data from the three major populations genotyped in HapMap Phase II (38). For this latter analysis, we first removed SNPs from two regions known to have strong linkage disequilibrium (major histocompatibility complex, the polymorphic chromosome 8 inversion) and then selected independent SNPs, required to have $r^2 < 0.2$ over a shifting window of 500 kb, yielding ~98 K SNPs. Principal components were selected based on a scree plot. In addition, cluster plots of called genotypes by allele intensities for SNPs showing association with outcomes were visually examined to ensure appropriate genotype calling. Imputation of an additional 1,677,236 ungenotyped autosomal SNPs was performed using release 22 Phase II CEU HapMap data (MACH v 1.0.16) and were analyzed using genotype probabilities and an additive genetic model (MACH2QTL v 1.04) (38,39). Genomic control lambda for mean A1C separately in CON, INT, and combined (COMB) treatment groups were all 1.01.

Statistical analysis. We performed a five-stage analysis (supplementary Fig. 6). Given the level of multiple hypothesis testing, we required P values $< 5 \times 10^{-8}$ for genome-wide significance in either the CON, INT, or COMB groups (40) and one-sided $P < 0.05$ for confirmation in the other treatment group with the same direction (41).

Stage 1 consisted of a genome-wide association study of the intra-individual mean A1C during DCCT, undertaken separately in each treatment group (667 CON individuals, 637 INT subjects) as well as in COMB including treatment as a covariate (Table 1). For this initial screen, we calculated the mean A1C from values obtained at eligibility screening, baseline, and up to 38 quarterly visits during DCCT for the conventional group. For INT and COMB, we excluded data obtained during the introduction of intensive therapy in the first year of DCCT. These means were converted to normal scores and tested for association with genotypes at each SNP using a 2df model, which does not assume a particular genetic model. Imputed SNPs were analyzed in a linear regression framework using the dosage of the imputed genotypes.

In stage 2, to identify loci that did not meet genome-wide significance using the mean A1C, but did so using the repeated measures, we tested the top SNPs from stage 1 ($P < 10^{-4}$) using linear regression mixed models including all of the available longitudinal A1C measures. In the intensive group, we used monthly values. We analyzed the natural log transformation of the A1C values with genotypes as a categorical variable and the time from DCCT eligibility as a continuous covariate, specifying random intercept and slope, and assumed an autoregressive moving average (1,1) covariance structure (because it had

TABLE 2
Results for the mean A1C in either the conventional, intensive, or combined treatment groups (stage 1) for any SNP with $P < 10^{-6}$

Chromosome	SNP	Position	Mean A1C	P	Common homozygote		Heterozygote		Rare homozygote		Minor allele frequency	Missing data*	Hardy-Weinberg equilibrium P	Location of SNP to the nearest gene
					Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n				
Conventional treatment group														
9	rs10810632	16,779,024	3.7	10^{-7}	8.96 \pm 1.22	85	9.63 \pm 1.22	86	9.61 \pm 1.23	5	10.63 \pm 1.35	C	T	Intron 1 BNC2
9	rs6475082	16,779,436	7.4	10^{-7}	8.96 \pm 1.22	86	9.61 \pm 1.23	5	10.63 \pm 1.35	5	10.63 \pm 1.35	G	A	Intron 1 BNC2
9	rs4961760	16,779,878	7.1	10^{-7}	8.96 \pm 1.22	87	9.61 \pm 1.22	5	10.63 \pm 1.35	5	10.63 \pm 1.35	C	T	Intron 1 BNC2
10	rs1358030	108,113,589	5.4	10^{-9}	8.71 \pm 1.15	307	9.24 \pm 1.25	90	9.44 \pm 1.24	90	9.44 \pm 1.24	C	T	3' SORCS1
18	rs163061	22,727,740	9.7	10^{-7}	8.83 \pm 1.17	259	9.29 \pm 1.26	35	9.62 \pm 1.43	35	9.62 \pm 1.43	G	C	3' C18orf16
Intensive treatment group														
15	rs493218	51,277,554	5.4	10^{-7}	7.31 \pm 0.96	120	7.01 \pm 0.89	11	6.38 \pm 0.69	11	6.38 \pm 0.69	C	T	3' WDR72
15	rs572221	51,291,924	6.8	10^{-7}	7.31 \pm 0.96	120	7.01 \pm 0.89	11	6.38 \pm 0.69	11	6.38 \pm 0.69	A	G	3' WDR72
15	rs690271	51,291,964	5.1	10^{-7}	7.31 \pm 0.96	121	7.01 \pm 0.88	11	6.38 \pm 0.69	11	6.38 \pm 0.69	A	G	3' WDR72
15	rs566369	51,295,884	5.1	10^{-7}	7.31 \pm 0.96	121	7.01 \pm 0.88	11	6.38 \pm 0.69	11	6.38 \pm 0.69	A	G	3' WDR72
15	rs482541	51,296,486	5.1	10^{-7}	7.31 \pm 0.96	121	7.01 \pm 0.88	11	6.38 \pm 0.69	11	6.38 \pm 0.69	A	G	3' WDR72
Combined treatment groups														
5	rs286405	35,613,014	6.7	10^{-7}	7.99 \pm 1.32	631	8.18 \pm 1.48	233	8.55 \pm 1.68	233	8.55 \pm 1.68	C	T	5' FLT23577
10	rs1358030	108,113,589	2.2	10^{-9}	7.93 \pm 1.32	574	8.35 \pm 1.57	183	8.48 \pm 1.53	183	8.48 \pm 1.53	C	T	3' SORCS1

A1C values are %. Mean = mean A1C value, SD = standard deviation of A1C values. A1 = minor, A2 = major allele. *Number of individuals with missing genotype data. Asymptotic P values from the 2df normal score test. Position is the nucleotide location from build 36.3.

the lowest Akaike information criterion [AIC]). Association of A1C with covariates and principal components from the Eigenstrat analysis was tested by linear regression, with results based on type 3 sum of squares (SAS PROCs REG and MIXED v9.1.3, Cary, NC).

In stage 3, SNPs achieving, or close to, genome-wide significance in stage 2 were tested for association with the repeated mean daily glucose, as well as C-peptide measured at eligibility screening for DCCT.

In stage 4, we analyzed the same SNPs with COMB A1C repeated measures, including treatment group as a covariate, as well as testing for interaction between each SNP and treatment.

Finally, since glycemia is a major risk factor for many diabetes complications, in stage 5, we tested the top A1C-associated SNPs for association with the following: the prevalence of coronary calcium, confirmed clinical neuropathy, hypoglycemia, as well as the time to event from DCCT baseline for three retinal and two renal outcomes (supplementary Table 2). This was done separately in each treatment group.

GoKinD. The Genetics of Diabetes in Kidneys (GoKinD) study was used to replicate associations with A1C and nephropathy. GoKinD is a case-extreme control study of renal disease in type 1 diabetes (42). Renal case and control subjects were defined as described elsewhere (42). Because, as expected the single cross-sectional A1C was significantly higher in renal case subjects than control subjects, the associations with A1C were analyzed separately. In addition, patients with a pancreas transplant were excluded from the A1C analysis. Genotype data from the Affymetrix SNP 5.0 were used (43) and imputed to HapMap phase 2 (release 24). Analyses were restricted to subjects with Caucasian ancestry as determined by genetic analysis and in whom appropriate genotype quality was observed.

MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium). We determined if any of the SNPs associated with A1C in DCCT were also associated with A1C in nondiabetic individuals. For this purpose, we obtained meta-analysis results for A1C from 23 genome-wide association studies including 27,589–36,585 (depending on the SNP studied) nondiabetic individuals of European descent (56) (see supplementary Methods).

RESULTS

To examine familial aggregation of A1C in type 1 diabetes, use of the A1C measured at eligibility for DCCT probands and A1C from sibs with type 1 diabetes (measured either during the DCCT family study [44] or the Epidemiology of Diabetes Interventions and Complications [EDIC] genetics study [33]) revealed significant familial correlation (Spearman rank correlation = 0.33, $P = 1 \times 10^{-3}$, $n = 95$ pairs, supplementary Fig. 14).

Stage 1: genome-wide association study of mean A1C. To address potential population stratification bias, we tested for association of mean A1C with principal components from Eigenstrat. When tested either individually or together, none of the three principal components were associated with mean A1C.

In the CON analysis, a single SNP (rs1358030, $P = 5 \times 10^{-9}$) on chromosome 10q25.1 met criteria for genome-wide significance (Table 2, supplementary Figs. 2 and 3), with five other SNPs within a 35-kb region having $P < 10^{-2}$ (Fig. 1). SNPs in two other chromosomal regions also attained evidence for association close to the genome-wide significance criteria in CON: one on chromosome 9 (rs10810632, $P = 4 \times 10^{-7}$), with eight other SNPs within a 20-kb interval (supplementary Fig. 11), and another on chromosome 18 (rs163061, $P = 1 \times 10^{-6}$), with no other associated SNPs. At each of the top SNPs in these three regions, the genotype-specific means were consistent with an additive model in which individuals carrying the rare allele had higher A1C (Table 2).

In the INT analysis, five SNPs on chromosome 15 had P values of 5×10^{-7} (Table 2, supplementary Figs. 8 and 13). The COMB analysis detected an additional SNP on chromosome 5, as well as the top CON chromosome 10 SNP (Table 2, supplementary Fig. 10). Analysis of imputed ungenotyped SNPs did not reveal any additional loci that met genome-wide significance using the mean A1C. Results for all SNPs are

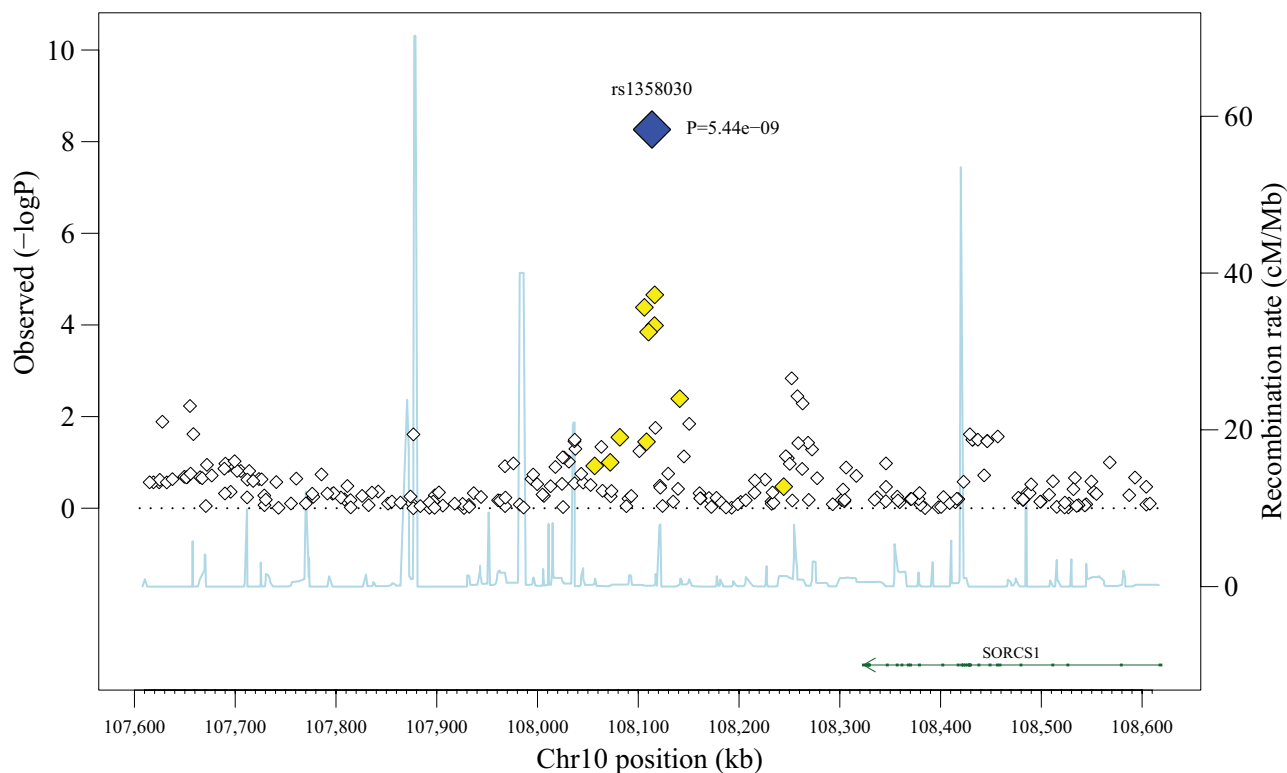


FIG. 1. Association results for mean A1C levels in the conventional treatment group at a 500-kb region surrounding rs1358030 (*SORCSI*). On the left *y*-axis is the $-\log_{10}(P)$ value for each SNP genotyped. On the right *y*-axis and the line is the recombination rate estimated from our data. The annotated genes in the region are indicated along the bottom of the figure. SNPs are colored based on their linkage disequilibrium with the most significant SNP, i.e., rs1358030, where black indicates the index SNP, light grey indicates $0.2 < r^2 < 0.5$, and white indicates $r^2 \leq 0.2$ (see the online version of the figure for colors where blue indicates the index SNP and yellow indicates $0.2 < r^2 < 0.5$).

available from http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000086.v1.p1.

Stage 2: repeated A1C measures. We selected the top SNPs from stage 1 according to a P value $< 10^{-4}$ and analyzed each of these 233 SNPs by mixed models using all the repeated measures. In CON, chromosome 9 and 10 SNPs from stage 1 became more significant (Table 3). In the INT group, however, there was little improvement for chromosome 15 SNPs over the analysis of the mean value (Table 3). In COMB (Table 3), three additional SNPs in a region on chromosome 14 approached genome-wide significance ($P = 3 \times 10^{-7}$, supplementary Fig. 12). Analysis of imputed SNPs did not provide evidence for additional loci meeting genome-wide significance (Table 3). Visual inspection of cluster plots for the SNPs in Tables 2 and 3 confirmed appropriate genotype calling (for rs1358030, see supplementary Fig. 15). Of the loci from the literature that were associated with glycemia in nondiabetic individuals, only rs13266634 (*SLC30A8*) was marginally associated with A1C in the DCCT, this being in the CON group ($P = 0.03$). This effect is in the same direction as the effect on A1C in nondiabetic individuals (28) and the effect on risk for type 2 diabetes (45) (N.B., supplementary Table 1).

Stage 3: repeated mean daily glucose measures and C-peptide. Analysis of the 13 SNPs in Table 3, with repeated mean daily glucose measures from all quarterly measures during DCCT in the CON group, revealed associations with SNPs in the chromosome 9 and 10 regions, consistent in direction with their effect on A1C (Table 4). There was also evidence for association of the chromosome 10 SNP with mean daily glucose in the INT group (Table 4). This argues that SNP variation at these loci

influences A1C through effects on glucose. None of the SNPs from Table 3 were associated with C-peptide measured at eligibility screening for DCCT ($P > 0.05$).

Stage 4: repeated A1C measures in both treatment groups with SNP treatment interaction. Testing for SNP by treatment interaction provided strong evidence for differences in the SNP associations between CON and INT at the regions on chromosomes 9 ($P = 1 \times 10^{-3}$) and 15 ($P = 3 \times 10^{-4}$), weaker on chromosome 10 ($P = 0.01$), but no evidence on chromosome 14 ($P = 0.25$, Table 4).

Stage 5: association of the 13 SNPs associated with A1C with complications. We then tested the A1C-associated SNPs (Table 3) for association with complications in the same subjects and found several biologically consistent associations of SNPs on chromosomes 9 and 10. The chromosome 10 signal, rs1358030, was associated with both time to persistent microalbuminuria ($P = 0.05$) and severe nephropathy ($P = 0.03$) in CON (Table 5 and supplementary Table 3). In COMB, rs1358030 was associated with the prevalence of coronary calcium ($P = 4 \times 10^{-3}$, supplementary Table 4). Even more striking, was that in CON, this SNP was associated with hypoglycemia either requiring medical assistance ($P = 1 \times 10^{-4}$) or resulting in coma or seizure ($P = 1 \times 10^{-3}$, Table 5 and supplementary Table 5). Inclusion of the mean DCCT A1C as a covariate in these later models reduced the association of the SNP with hypoglycemia ($P = 0.03$ and 0.02 , respectively), consistent with an effect being mediated through glycemic control (supplementary Table 5). Similarly, in CON, SNPs in the chromosome 9 locus were associated with the following: time to persistent microalbuminuria ($P = 5 \times 10^{-4}$), severe nephropathy ($P =$

TABLE 3
Loci with $P \leq 10^{-7}$ in the conventional, intensive, or combined treatment groups using repeated AIC values (stage 2)

Chromosome	SNP	Position	Repeated AIC P value	Mean AIC P value	Mean AIC by genotype from repeated analysis						Minor allele frequency	Missing data*	Hardy-Weinberg equilibrium	Location of SNP to nearest gene
					C homo (SE)	Het (SE)	R homo (SE)	A1	A2					
Conventional treatment group														
9	rs10810632	16,779,024	9.0×10^{-8}	3.7×10^{-7}	8.83 (0.05)	9.50 (0.13)	10.50 (0.63)	C	T	0.08	1	0.70	Intron 1 BNC2	
9	rs6475082	16,779,436	1.8×10^{-7}	7.4×10^{-7}	8.83 (0.05)	9.48 (0.13)	10.50 (0.63)	G	A	0.08	1	0.71	Intron 1 BNC2	
9	rs4961760	16,779,878	1.6×10^{-7}	7.1×10^{-7}	8.83 (0.05)	9.48 (0.13)	10.50 (0.63)	C	T	0.08	0	0.71	Intron 1 BNC2	
9	rs2254193	16,791,850	2.0×10^{-7}	1.1×10^{-6}	8.84 (0.05)	9.57 (0.15)	10.47 (0.70)	C	A	0.07	8	0.67	Intron 1 BNC2	
10	rs1358030	108,113,589	6.9×10^{-10}	5.4×10^{-9}	8.58 (0.07)	9.12 (0.07)	9.32 (0.13)	C	T	0.36	2	0.12	3' SORCSI	
Intensive treatment group														
15	rs493218	51,277,554	5.2×10^{-7}	5.4×10^{-7}	7.26 (0.04)	6.99 (0.07)	6.42 (0.22)	C	T	0.11	2	0.20	3' WDR72	
15	rs572221	51,291,924	6.6×10^{-7}	6.8×10^{-7}	7.26 (0.04)	6.99 (0.07)	6.42 (0.22)	A	G	0.11	2	0.25	3' WDR72	
15	rs690271	51,291,964	5.0×10^{-7}	5.1×10^{-7}	7.26 (0.04)	6.99 (0.07)	6.42 (0.22)	A	G	0.11	0	0.26	3' WDR72	
15	rs566369	51,295,884	5.0×10^{-7}	5.1×10^{-7}	7.26 (0.04)	6.99 (0.07)	6.42 (0.22)	A	G	0.11	1	0.26	3' WDR72	
15	rs482541	51,296,486	5.0×10^{-7}	5.1×10^{-7}	7.26 (0.04)	6.99 (0.07)	6.42 (0.22)	A	G	0.11	1	0.26	3' WDR72	
Combined treatment groups														
10	rs1358030	108,113,589	3.8×10^{-10}	2.2×10^{-9}	7.84 (0.04)	8.16 (0.04)	8.36 (0.08)	C	T	0.36	2	0.12	3' SORCSI	
14	rs11624318	94,375,765	2.7×10^{-7}	7.6×10^{-6}	8.07 (0.04)	8.11 (0.05)	7.43 (0.12)	A	C	0.21	1	0.28	5' GSC	
14	rs11160219	94,405,244	4.3×10^{-7}	3.4×10^{-5}	8.10 (0.04)	8.08 (0.05)	7.47 (0.11)	A	G	0.24	1	0.49	5' GSC	
14	rs8007115	94,407,241	5.4×10^{-7}	4.1×10^{-5}	8.10 (0.04)	8.07 (0.05)	7.47 (0.11)	T	C	0.23	3	0.59	5' GSC	

Mean AIC values are results from stage 1. Longitudinal repeated AIC P values are the results from stage 2. C homo, Het, and R homo are least square means of AIC in the common homozygote, heterozygote and rare homozygote genotype groups, respectively, with SEs in parentheses, back-transformed from analysis of repeated lnAIC. NB. The statistical results for the repeated AIC were based on normal scores. A1 = minor, A2 = major allele. *Number of individuals with missing genotype data. Position is the nucleotide location from build 36. Location relative to gene is from dbSNP build 129.

TABLE 4
Loci for which there is evidence for association using repeated A1C values (stages 3 and 4)

Chromosome	SNP	Position	Stage 2: repeated A1C P			Stage 3: repeated daily glucose P			Stage 3: mean glucose by genotype (mg/dl)			Stage 4: repeated A1C P value (COMB = CON +INT)	
			CON	INT	CON	INT	COMB	C homo (SE)	Het (SE)	R homo (SE)	SNP P	SNP treatment interaction	
9	rs10810632	16,779,024	9.0 × 10 ⁻⁸	0.44	9.7 × 10 ⁻³	0.62	0.52	216 (2)*	226 (5)*	259 (25)*	9.2 × 10 ⁻³	7.9 × 10 ⁻⁴	
9	rs6475082	16,779,436	1.8 × 10 ⁻⁷	0.44	0.011	0.62	0.52	216 (2)*	225 (5)*	259 (25)*	0.011	1.1 × 10 ⁻³	
9	rs4961760	16,779,878	1.6 × 10 ⁻⁷	0.44	0.012	0.62	0.53	216 (2)*	225 (5)*	259 (25)*	0.010	1.1 × 10 ⁻³	
9	rs2254193	16,791,850	2.0 × 10 ⁻⁷	0.67	8.3 × 10 ⁻³	0.50	0.34	216 (2)*	229 (6)*	253 (27)*	0.011	3.1 × 10 ⁻³	
10	rs1358030	108,113,589	6.9 × 10 ⁻¹⁰	0.012	2.7 × 10 ⁻⁵	5.8 × 10 ⁻³	1.5 × 10 ⁻⁶	208 (3)*	223 (3)*	228 (5)*	3.8 × 10 ⁻¹⁰	0.013	
14	rs11624318	94,375,765	9.4 × 10 ⁻⁴	2.9 × 10 ⁻³	0.40	0.035	0.025	180 (1)†	182 (2)‡	170 (4)‡	2.7 × 10 ⁻⁷	0.95	
14	rs11160219	94,405,244	1.1 × 10 ⁻⁵	0.023	0.37	0.58	0.49	181 (1)†	180 (2)‡	176 (4)‡	4.3 × 10 ⁻⁷	0.25	
14	rs8007115	94,407,241	2.2 × 10 ⁻⁵	0.017	0.38	0.78	0.49	181 (1)†	180 (2)‡	176 (4)‡	5.4 × 10 ⁻⁷	0.37	
15	rs493218	51,277,554	0.77	5.2 × 10 ⁻⁷	0.32	0.10	0.074	149 (1)†	145 (2)‡	142 (7)†	8.9 × 10 ⁻³	2.4 × 10 ⁻⁴	
15	rs572221	51,291,924	0.83	6.6 × 10 ⁻⁷	0.26	0.088	0.056	149 (1)†	145 (2)‡	142 (7)†	8.3 × 10 ⁻³	3.6 × 10 ⁻⁴	
15	rs690271	51,291,964	0.83	5.0 × 10 ⁻⁷	0.26	0.085	0.050	149 (1)†	145 (2)‡	142 (7)†	7.6 × 10 ⁻³	3.4 × 10 ⁻⁴	
15	rs566369	51,295,884	0.82	5.0 × 10 ⁻⁷	0.26	0.085	0.050	149 (1)†	145 (2)‡	142 (7)†	7.6 × 10 ⁻³	3.4 × 10 ⁻⁴	
15	rs482541	51,296,486	0.82	5.0 × 10 ⁻⁷	0.26	0.085	0.050	149 (1)†	145 (2)‡	142 (7)†	7.6 × 10 ⁻³	3.4 × 10 ⁻⁴	

Data are presented only for SNPs that were close to or met genome-wide significance ($P < 5 \times 10^{-8}$) from stage 2 using the longitudinal repeated measures in the conventional treatment group and either nominal significance in stage 3 for the intensive group, or reach genome-wide significance when both treatment groups were combined. For the intensive group only analysis, monthly measures were used. For the CON + INT analysis, only quarterly measures were used for the intensive group, so that they would be comparable to the conventional group. C homo, Het, and R homo are least square means of daily mean of the back-transformed Ln(glucose) in the common homozygote, heterozygote, and rare homozygote genotype groups, respectively, in one of the following groups: *CON, †INT, ‡COMB. NB. The statistical analysis of the glucose measures was performed using normal scores.

2×10^{-3} , Table 6 and supplementary Table 3), mild retinopathy ($P = 2 \times 10^{-4}$), clinically significant macular edema ($P = 1 \times 10^{-3}$), and severe retinopathy ($P = 2 \times 10^{-3}$, Table 6 and supplementary Table 6) as well as hypoglycemia requiring assistance ($P = 4 \times 10^{-3}$, Table 6 and supplementary Table 5). More modest results were obtained for SNPs in the chromosome 14 and 15 regions for time to mild retinopathy ($P = 0.01$ and 0.02 , respectively, supplementary Table 6). The directions of these associations at these three regions were consistent with their effects on A1C, i.e., the genotype with higher A1C had higher risk of renal, retinal, cardiac, and neuropathic complications, while having lower risk of hypoglycemia (Tables 5 and 6).

Confirmation of rs1358030 associated with A1C in GoKinD. We then tested the top 13 SNPs that were identified from the four regions for A1C in DCCT for association with A1C in GoKinD separately by case-control status. The mean \pm SD A1C in case subjects without pancreas transplant was 8.29 ± 1.56 ($n = 531$), while in control subjects, it was 7.47 ± 1.15 ($n = 851$). There was nominal evidence for association of rs1358030 with A1C in control subjects, but not case subjects ($P = 0.01$ and $P = 0.8$, respectively). In the control subjects, the direction of the effect at rs1358030 is consistent with that in DCCT, with higher A1C values in individuals with more copies of the rare allele (supplementary Table 7). Testing association of the same 13 SNPs with renal disease, separately in 795 case subjects and 856 control subjects, revealed no significant evidence for association at any of the 13 SNPs ($P \geq 0.1$, supplementary Table 8).

Confirmation of BNC2 association with A1C in nondiabetic individuals. To determine if there was evidence for association of the SNPs identified in DCCT with A1C in nondiabetic individuals, we obtained summary statistics from an analysis of A1C in >27.5 K nondiabetic individuals, carried out by the MAGIC investigators. There was nominal evidence consistent with association at SNPs in the chromosome 9 region (e.g., rs10810632, Table 7), which is in the same direction as the association observed with A1C in the DCCT CON group (Tables 2 and 3).

DISCUSSION

Using repeated longitudinal measures of A1C in the DCCT and a multistage analysis, we identified a novel locus (rs1358030) with genome-wide significant association in the conventional treatment group. This locus is also associated with mean glucose during DCCT, arguing against a specific effect on glycation of hemoglobin or red cell survival. Analysis of repeated A1C in the intensive group supported this locus at $P = 0.012$, with additional supportive evidence in renal control subjects from the GoKinD study ($P = 0.01$).

None of the SNPs that were associated A1C were found to be associated with C-peptide at DCCT baseline. Important caveats to these negative results, however, are important to remember, since the DCCT was not an inception cohort of newly diagnosed type 1 diabetes, C-peptide levels were an inclusion criteria, and it has been shown that glycemic control influences persistence of C-peptide (1,35,46).

The SNP with the most significant association (rs1358030) is ~ 200 kb 3' from *SORCS1* (sortilin-related vacuolar protein sorting 10 domain containing receptor 1), the nearest annotated gene. Because there are prior data

TABLE 5
Association of rs1358030 (*SORCS1*) with complications in the DCCT/EDIC

Outcome	Conventional		Intensive	
	OR or HR (95% CI)	<i>P</i>	OR or HR (95% CI)	<i>P</i>
Hypoglycemia				
Requiring medical assistance	0.63 (0.50–0.80)	0.0001	0.89 (0.72–1.10)	0.28
Resulting in coma/seizure	0.60 (0.44–0.81)	0.001	1.05 (0.84–1.31)	0.64
Retinopathy				
Mild nonproliferative retinopathy	1.15 (0.99–1.34)	0.07	1.16 (0.99–1.35)	0.07
Severe nonproliferative retinopathy	1.30 (1.04–1.63)	0.02	1.27 (0.92–1.77)	0.15
Clinically significant macular edema	1.16 (0.92–1.46)	0.22	1.10 (0.8–1.5)	0.57
Nephropathy				
Persistent microalbuminuria	1.25 (1.01–1.56)	0.04	1.25 (0.93–1.67)	0.14
Severe nephropathy	1.39 (1.04–1.87)	0.03	1.00 (0.61–1.64)	0.99
Coronary calcium ($\beta \pm$ SE)	0.83 \pm 0.36	0.05	0.68 \pm 0.35	0.02
Confirmed clinical neuropathy	1.32 (1.01–1.73)	0.05	1.08 (0.81–1.43)	0.19

See supplementary Table 2 for definition of complications outcomes. Analysis was performed using additive coding of genotype, with the direction expressed as the number of copies of the C alleles (compared with the T allele). HR, hazard ratio; OR, odds ratio.

supporting the involvement of this gene in glycemic traits, we focus discussion below on *SORCS1*. A previous study mapped a major locus for fasting plasma insulin levels to a 242-kb interval containing only the promoter, first exon, and most of the first intron of *Sorcs1*, using a conditioned cross between mice carrying the “obese” Leptin mutation. Between the two strains, 46 sequence differences were identified in *Sorcs1*, including three nonsynonymous changes, making it difficult to ascribe phenotypic differences to a specific nucleotide change (47). Differences in *Sorcs1* expression in islets between the two strains were documented (47). Independent data supporting *SORCS1* as a locus for glycemic traits comes from a mapping study that determined that *Sorcs1* was the only gene in a major locus for post-intraperitoneal glucose tolerance tests in a rat model of diabetes (48). There was modest evidence for association of SNPs in *SORCS1*, with a single measure of fasting insulin in 574 nondiabetic Mexican Americans from 102 families ($P = 4 \times 10^{-3}$). Further, in the Framingham Heart Study, there was nominal association of rs1416406, which is \sim 120 kb 5' of *SORCS1*, with fasting insulin, insulin sensitivity index, and insulin resistance ($P = 0.02$ – 0.005) (49). However, this SNP was not statistically significantly associated ($P > 0.4$) with type 2 diabetes in four replication studies. Importantly, neither of the SNPs from these two studies (49,50) are in strong linkage disequilibrium with rs1358030. Further, rs1358030 is not in

strong linkage disequilibrium with any other SNP genotyped in HapMap phase 2 or 3 ($r^2 < 0.5$, supplementary Fig. 4) (38), arguing that it is either the etiological variant or is in strong linkage disequilibrium with a causal variant not genotyped in HapMap.

The regions associated with A1C on chromosomes 9 (BNC2), 14 (WDR72), and 15 (*GSC*) have not been previously shown to be associated with glycemic traits in humans or animals. It is interesting to note that there is evidence for confirmation of the BNC2 region in a large meta-analysis of A1C from nondiabetic individuals, suggesting that some of the loci influencing glycemic control in type 1 diabetes also influence glycemia in nondiabetic individuals, consistent with twin studies (14). The associated SNPs in BNC2 are close to *LOC648570*, a hypothetical gene.

Because of the larger “environmental” effect of INT during DCCT, mean A1C was \sim 1.8% lower than in the CON group (Table 1), so INT cannot be considered a straightforward replication group for loci identified in CON (Table 4). Despite the concern that in the INT, subjects adjusted insulin dose to achieve treatment aims, we specifically show at the four loci that inclusion of insulin dose as a covariate does not diminish the SNP association with A1C (supplementary Table 9). If a genetic association depends on the level of A1C, then evidence for association may differ between the two groups. Moreover, overestimation

TABLE 6
Association of rs10810632 (*BNC2*) with complications in the DCCT/EDIC

Outcome	Conventional		Intensive	
	OR or HR (95% CI)	<i>P</i>	OR or HR (95% CI)	<i>P</i>
Hypoglycemia				
Requiring medical assistance	0.49 (0.30–0.80)	0.004	1.02 (0.70–1.47)	0.94
Resulting in coma/seizure	0.60 (0.32–1.10)	0.10	0.89 (0.60–1.33)	0.58
Retinopathy				
Mild nonproliferative retinopathy	1.73 (1.31–2.27)	0.0002	1.02 (0.77–1.35)	0.90
Severe nonproliferative retinopathy	1.83 (1.29–2.62)	0.002	0.61 (0.3–1.25)	0.15
Clinically significant macular edema	1.87 (1.31–2.67)	0.001	0.97 (0.56–1.69)	0.92
Nephropathy				
Persistent microalbuminuria	1.84 (1.31–2.59)	0.001	0.80 (0.46–1.41)	0.43
Severe nephropathy	1.85 (1.19–2.87)	0.01	0.32 (0.08–1.31)	0.06
Coronary calcium ($\beta \pm$ SE)	0.64 \pm 0.62	0.30	1.34 \pm 0.57	0.02
Confirmed clinical neuropathy	1.64 (1.00–2.68)	0.05	0.69 (0.40–1.20)	0.6

See legend to Table 5. Additive coding of genotype, with the direction expressed as the number of copies of the C alleles (compared with the T allele).

TABLE 7
Association results of top 13 SNPs from DCCT with A1C in the MAGIC study of nondiabetic individuals

Chromosome	Position	SNP	Allele 1	Allele 2	Effect	SE	P	Sample size
<i>BNC2</i> gene region								
9	16,779,024	rs10810632	t	c	-0.0220	0.0065	7.1×10^{-4}	36,446
9	16,779,436	rs6475082	a	g	-0.0235	0.0064	2.7×10^{-4}	36,557
9	16,779,878	rs4961760	t	c	-0.0194	0.0065	0.0030	35,837
9	16,791,850	rs2254193	a	c	-0.0235	0.0066	3.5×10^{-4}	36,458
<i>SORCS1</i> gene region								
10	108,113,589	rs1358030	a	g	0.0014	0.0036	0.70	35,304
<i>GSC</i> gene region								
14	94,375,765	rs11624318	a	c	0.0016	0.0042	0.71	35,801
14	94,405,244	rs11160219	a	g	-0.0018	0.0044	0.68	32,898
14	94,407,241	rs8007115	t	c	-0.0012	0.0045	0.79	27,589
<i>WDR72</i> gene region								
15	51,277,554	rs493218	t	c	-0.0015	0.0057	0.79	36,530
15	51,291,924	rs572221	a	g	0.0015	0.0057	0.79	36,561
15	51,291,964	rs690271	a	g	0.0014	0.0057	0.81	36,585
15	51,295,884	rs566369	a	g	0.0012	0.0057	0.83	36,575
15	51,296,486	rs482541	a	g	0.0010	0.0057	0.86	36,577
18	22,727,740	rs163061	c	g	-0.0048	0.0041	0.25	33,297

Allele 1 indicates the effect allele compared with allele 2 as the reference. Alleles are aligned to HapMap forward strand, but are not aligned in terms of minor or major allele. The A1C values were untransformed % [National Glycohemoglobin Standardization Program transformation: %A1C = $91.48 \times (A1C/Hb) + 2.152$], adjusted for age and sex.

of the SNP effects in one treatment group, due to the Winner's curse (51), could explain a SNP-by-treatment interaction (Table 4). Nevertheless, the findings on chromosome 10 in repeated measures analysis of the intensive group ($P = 0.012$) argue for an effect in both treatment groups, as well as GoKinD control subjects. The nonsignificant results in the GoKinD case subjects may be due to lower power (estimated to be 43%).

Importance of repeated A1C measures. To emphasize the importance of longitudinal measures in this study, we compared the results from the stage 1 (mean A1C analysis) with analysis of a single A1C measure (obtained at DCCT eligibility screening) at the most significant CON SNP (rs1358030). The latter produced nominal significance ($P = 0.027$) and accounted for only 0.61% of the variance in 1,304 individuals. This compares to 5.2% ($P = 5 \times 10^{-9}$) of the variance in the former analysis (noting however that this latter estimate may be upwardly biased [51]). We calculated that 6,580 subjects (i.e., approximately five times larger than DCCT) would be needed to detect a comparable effect using a single A1C measure ($\alpha = 5 \times 10^{-8}$, $1 - \beta = 0.8$) (52). These calculations, however, assume 1) the mean A1C values from a contemporary study would be similar to values at DCCT eligibility (mean = 9.0%) and 2) the genetic effect is similar across the range of A1C values. Current clinical studies in type 1 diabetes report lower mean A1C levels than that observed at DCCT eligibility (53). Further, in the conventional group, the mean A1C dropped from 9.1 to 7.8% during follow-up in EDIC (12). In addition, rs1358030 accounted for only 1.7% of the variance in mean A1C in the intensive group (compared with 5.2% in the conventional group), consistent with a possible relationship between effect size and A1C levels. Therefore, the combination of these two factors could result in even larger sample sizes being required for the same power to detect a genetic effect using a single A1C measure in a contemporary setting. This clearly emphasizes the advantage of exploiting longitudinal measures of variable traits such as A1C, as has been described in other studies (54).

In conclusion, although a major locus for A1C levels in type 1 diabetes was identified, a number of outstanding questions remain. These include evaluation in other groups: nonwhite individuals, type 1 diabetic subjects who do not meet DCCT eligibility criteria, and type 2 diabetic subjects. *SORCS1* is not a known susceptibility loci for either type 1 or type 2 diabetes, nor does it overlap with the loci identified for fasting glucose in nondiabetic individuals (Table 7) (20–23). Further, the etiological variants and mechanisms by which genetic variation influences glycemic control are unknown. Nonetheless, understanding of genetic factors that influence an individual's ability to control their A1C levels has potential application to clinical care and may provide insight into why patients on the same regimen have different A1C values. In the future, it may be possible to recommend different treatment or regimen approaches to subjects based on genotype to achieve similar targets. In the design and analysis of genetic studies, attempting to identify risk factors for long-term diabetic complications confounding by loci for glycemic control may be reduced by inclusion of longitudinal measures of A1C as covariates.

ACKNOWLEDGMENTS

The DCCT/EDIC Research Group is sponsored through research contracts from the National Institute of Diabetes, Endocrinology and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institutes of Health. S.B.B. held a Canadian Institutes of Health Research (CIHR) Senior Investigator award (2002–2007). A.D.P. holds a Canada Research Chair in the Genetics of Complex Diseases. This work received support from National Institute of Diabetes and Digestive and Kidney Diseases contract N01-DK-6-2204, National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-077510, R01-DK077489, and P60-DK20595 and support from the Canadian Network of Centres of Excellence in Mathematics and Genome Canada through the Ontario Genomics Institute. Clinical data and DNA from the DCCT/EDIC study will be made available through the National Institute of Diabetes and

Digestive and Kidney Diseases repository at <https://www.niddkrepository.org/niddk/home.do>. The GoKinD study sample collection was supported by the Juvenile Diabetes Research Foundation in collaboration with the Joslin Diabetes Center and George Washington University and by the U.S. Centers for Disease Control and Prevention.

No potential conflicts of interest relevant to this article were reported.

The authors are grateful to the subjects in the DCCT/EDIC cohort for their long-term participation.

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