Genetic Risk Factors for CVD in Type 1 Diabetes: The DCCT/EDIC Study

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OBJECTIVE

The role of genetic factors in the risk of cardiovascular disease (CVD) for patients with type 1 diabetes (T1D) remains unknown. We therefore examined whether previously identified genetic factors for coronary artery disease (CAD) are associated with the risk of CVD above and beyond established demographic and clinical factors in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study.

RESEARCH DESIGN AND METHODS

Polygenic risk scores (PRS) and individual genetic variants identified in previous studies were obtained from genome-wide genotyping performed in 1,371 DCCT/EDIC participants. Two composite CVD outcomes were considered: major adverse cardiovascular events (MACE) (CVD death or nonfatal myocardial infarction [MI] or stroke) and any CVD (MACE plus confirmed angina, silent MI, revascularization, or congestive heart failure). Cox proportional hazards models assessed the association between the genetic factors and the risk of CVD with adjustment for other factors (including age, lipids, blood pressure, and glycemia).

RESULTS

CAD PRS was strongly associated with the subsequent risk of any CVD (42% and 38% higher risk per 1-SD increase in unadjusted and fully adjusted models, respectively; $P < 0.0001$) and with the risk of MACE (50% and 40% higher risk per 1-SD increase in unadjusted and fully adjusted models, respectively; $P < 0.0001$). Several individual single nucleotide polymorphisms were also nominally associated with the risk of any CVD and MACE.

CONCLUSIONS

Genetic factors are associated with the risk of subsequent CVD in individuals with T1D above and beyond the effect of established risk factors such as age, lipids, blood pressure, and glycemia.

Despite progress in diabetes, cardiovascular risk factor, and cardiovascular disease (CVD) management over the past several decades, the risk for CVD remains higher in individuals with type 1 diabetes (T1D) compared with age-matched individuals without diabetes (1,2). We previously reported that an average of 6.5 years of intensive diabetes therapy during the Diabetes Control and Complications Trial (DCCT) substantially reduced the risk of CVD compared with conventional therapy during the 10 years of follow-up in the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study (3). After adjustment for other

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*A complete list of the members of the DCCT/EDIC Research Group can be found in N Engl J Med 2017;376:1507–1516.

traditional CVD risk factors, multivariable risk factor analyses identified elevations in HbA_{1c} levels as the second strongest risk factor, after age, for CVD events in the DCCT/EDIC cohort (4).

In the general population, several cross-sectional studies have identified associations between both individual genetic variants and genome-wide polygenic risk scores (PRS) and the presence of coronary artery disease (CAD) (5). The role of genetic factors for CAD in T1D remains unknown. Herein, we report the evaluation of such genetic factors as risk factors for CVD in participants with T1D in DCCT/EDIC. Of particular importance, we also evaluated whether the associations between genetic factors and CVD are independent of other established and putative risk factors that have been measured over more than 30 years.

RESEARCH DESIGN AND METHODS

The DCCT and EDIC studies have previously been described in detail (4,6,7). Briefly, the DCCT randomized 1,441 individuals with T1D to receive either conventional diabetes therapy, aimed at maintaining freedom from symptoms of hyper- and hypoglycemia, or to intensive therapy, aimed at safely achieving glycemia as close to nondiabetic levels as safely possible. At baseline, individuals at increased risk for CVD because of high cholesterol or blood pressure (BP) and individuals with established CVD or severe retinal or renal disease were excluded by study design. At the end of the DCCT in 1993, after an average of 6.5 years of follow-up, all participants were instructed in intensive therapy and referred to their personal health care providers for subsequent diabetes care. In 1994, 97% of the surviving DCCT cohort enrolled in the observational EDIC study, and 94% continued to participate actively in annual study visits after >20 years of additional EDIC follow-up. The DCCT and EDIC protocols were approved by the institutional review boards of all participating centers, and all participants provided written informed consent.

The present analyses used all available data over ~30 years of combined DCCT/EDIC follow-up in the 1,371 DCCT/EDIC participants with available genome-wide genotyping data.

CVD Clinical Risk Factors

The results reported herein are based on data obtained at quarterly visits during DCCT and annual visits during EDIC. These included detailed medical histories, physical examinations (including measurements of height, weight, sitting BP, and pulse rate), and collections of blood and urine biospecimens. Recognized and putative CVD risk factors were evaluated by standardized methods (6,7). HbA_{1c} was obtained quarterly during DCCT and annually during EDIC. Fasting lipids (triglycerides and total and HDL cholesterol) were obtained and measured in the central laboratory annually during DCCT and every other year during EDIC. LDL cholesterol (LDLc) was calculated using the Friedewald equation (8). Albumin excretion rates were measured from 4-h urine samples from DCCT at baseline through EDIC year 18 (2012) and from spot urine samples thereafter, with albumin excretion rates estimated using the ratio of urine albumin to creatinine concentrations (9). The use of antihypertensive medications was captured annually during EDIC. Pulse pressures were calculated as the difference between systolic BP (SBP) and diastolic BP, hypertension was defined as SBP/diastolic BP ≥140/90 mmHg or antihypertensive medication use, and hyperlipidemia was defined as LDLc ≥130 mg/dL or lipid-lowering medication use. Electrocardiograms (read centrally) were obtained at DCCT baseline, every 2 years during DCCT, and at DCCT closeout and annually during EDIC.

Genetic Risk Factors

Genotypes of the DCCT/EDIC study participants were generated with the Illumina HumanCoreExome BeadArrays, and imputations were performed for ungenotyped autosomal single nucleotide polymorphisms (SNPs) using the phase 3 (version 5) release of the 1000 Genomes data as a reference panel (10,11). Genomic coordinates, SNP locations, and labels are based on Human Genome (GRC37/hg19).

For detection of population structure and potential outliers, principal components analysis was performed. Cryptically related individuals and/or sample mix-ups were identified using the identical-by-state estimates between all pairs of individuals, and one from each pair was removed (11).

Data cleaning was performed by using the quality control criteria of SNP call rate (≥95%), subject call rate (≥98%), and Hardy-Weinberg equilibrium ($P \geq 1E{-}6$). Moreover, SNPs with 20% (or 5% for rare variants) difference in minor allele frequency compared with the 1000 Genomes EUR superpopulation were excluded. Genotyping, quality control, and imputation were performed at a single site at the University of Virginia.

Two sets of genetic variants were used to investigate their associations with CVD and major adverse cardiovascular events (MACE) (Supplementary Fig. 3). First, Khera et al. (5) derived, validated, and tested robust PRS for CAD in the UK Biobank baseline data by aggregating the information from ~6.6 million SNPs across the genome, with correction for linkage disequilibrium. Additional details on the calculation of the PRS for CAD are available on the CVD knowledge portal (https://kp4cd.org/dataset_downloads/mi). Second, Nelson et al. (12) used the UK Biobank data (July 2015 interim release) and examined two CAD phenotypes, SOFT CAD (n = 10,801 cases) and HARD CAD (n = 6,482 cases). SOFT CAD cases included self-reported angina and other chronic heart disease, while HARD CAD cases included evidence of myocardial infarction (MI) and/or revascularization. Separate meta–genome-wide association studies were conducted for each CAD phenotype using the UK Biobank and the CARDIoGRAMplusC4D 1000 Genomes (13) and the MIGen/CARDIoGRAM Exome chip (14). The meta–genome-wide association study of SOFT CAD included 71,602 cases and 260,875 controls and identified 78 genome-wide significant loci associated with CAD. In this study, the individual effects of those 78 SNPs were investigated for associations with CVD and MACE in DCCT/EDIC. In addition, we used the two genetic risk scores (GRS) derived from those 78 SNPs, one weighted by effect size and the other unweighted.

R (version 3.4.3) software (R Foundation) and PLINK2 software (15) were used for these calculations.

Table 1—Baseline characteristics of DCCT/EDIC participants overall and by CAD PRS tertile

		CAD PRS tertile		
	Overall (n = 1,371)	First (lowest) (n = 457)	Second (middle) (n = 457)	Third (highest) (n = 457)
Intensive group	49.2	46.8	52.1	48.6
Primary cohort	50.4	51.0	51.4	48.8
Male sex	52.7	51.6	55.6	51.0
Age, years	26.8 (7.1)	26.5 (7.1)	26.9 (7.1)	26.9 (7.1)
Duration, months	69.7 (49.7)	68.0 (48.1)	67.9 (49.5)	73.1 (51.5)
Currently smoking	18.6	17.1	19.5	19.3
SBP, mmHg	114.6 (11.3)	114.2 (11.4)	115.0 (11.0)	114.6 (11.5)
Diastolic BP, mmHg	73.1 (8.5)	72.8 (8.6)	73.1 (8.3)	73.3 (8.5)
HDLc, mg/dL	50.4 (12.2)	51.4 (13.1)	49.7 (11.7)	49.9 (11.8)
LDLc, mg/dL	109.4 (29.1)	105.4 (28.6)	108.3 (28.2)	114.6 (29.8)
Triglycerides, mg/dL	81.9 (48.2)	79.7 (45.1)	83.3 (56.3)	82.6 (42.1)
HbA_{1c}, %	8.9 (1.6)	8.9 (1.6)	8.8 (1.5)	9.0 (1.6)
HbA_{1c}, mmol/mol	73.8 (17.5)	73.8 (17.5)	72.7 (16.4)	74.9 (17.5)
AER, mg/24 h	15.9 (18.8)	14.5 (13.5)	16.1 (22.4)	16.9 (19.5)
Estimated GFR, mL/min/1.73 m^2	125.8 (13.9)	125.9 (14.1)	125.2 (13.5)	126.2 (14.1)

Data are given as percentages for binary factors and mean (SD) for quantitative factors. AER, albumin excretion rate; GFR, glomerular filtration rate.

Cardiovascular Events

CVD events were ascertained using annual medical histories and electrocardiograms. All CVD events were adjudicated using additional information documented in participants' nonstudy medical records by a committee masked to DCCT treatment groups and HbA_{1c} levels. The composite CVD outcome (CVD) was defined as time to the first occurrence of any of the following: CVD death, nonfatal MI, nonfatal stroke, subclinical myocardial infarction on ECG, angina confirmed by ischemic changes with exercise tolerance testing or by clinically significant coronary obstruction demonstrated by angiography, revascularization (with coronary angioplasty stenting or coronary artery bypass), or congestive heart failure (paroxysmal nocturnal dyspnea, orthopnea, or marked limitation of physical activity caused by heart disease) (16). A secondary CVD outcome, MACE, was defined as a first occurrence of CVD death, nonfatal MI, or nonfatal stroke. All CVD events that occurred prior to 18 May 2017, a total of ~30 years of study, were included in these analyses; participants without an event were censored at that time.

Statistical Analysis

Variables were fixed (e.g., sex) or time dependent (e.g., HbA_{1c}), with the latter captured either as the current (most recent) value or as the updated mean from baseline. An updated mean is the weighted average of prior values using weights proportional to the time interval between the measurements (i.e., 1/4 for quarterly visits during DCCT and 1 for annual visits during EDIC). The number of CVD events is reported along with the rate of events per 1,000 individuals at risk for 1 year (1,000 person-years [PYs]).

Separate Cox proportional hazards models assessed the associations between the genetic factors (one at a time) and the risk of CVD and MACE from DCCT baseline until event date. Unadjusted, minimally adjusted for age and mean updated HbA_{1c}, and fully adjusted models, as previously described (4), were evaluated. More specifically, the fully adjusted model for CVD included adjustments for age, time-weighted HbA_{1c}, (log) triglycerides, time-weighted SBP, time-weighted pulse, family history of MI, duration of T1D at DCCT baseline, ACE inhibitor use, and time-weighted LDLc. The fully adjusted model for MACE was further adjusted for age at DCCT baseline, time-weighted HbA_{1c}, smoking, current (log) triglycerides, time-weighted SBP, time-weighted pulse, duration of T1D at DCCT baseline, ACE inhibitor use, and current LDLc. The Akaike information criterion (IC) indicated the relative model quality (smaller values are better).

PRS were used both as categorical variables (using tertiles) and as quantitative risk factors (with results reported per 1 SD). Given their distribution (Supplementary Fig. 1), individual SNPs were analyzed as class variables, with three levels obtained by rounding their value to the closest integer (0, 1, or 2), and their association with the risk of outcomes was assessed in the Cox proportional hazards models using a 2 df test.

Separately for CVD and MACE, the genetic risk factors identified were then further evaluated jointly in multivariable models adjusted for clinical risk factors. Adjusted for age, the restricted mean survival time, with a time horizon of 30 years, was used to compare the average time to CVD and MACE between individuals with a PRS value in the third tertile versus individuals with a PRS value in the lower two tertiles combined, both on the additive scale and the time-loss ratio scale (17). The order in which the genetic risk factors (polygenic risk factors, n = 3; individual genetic variants, n = 78) were analyzed was prespecified, starting with the CAD PRS.

In addition, the performance of the three polygenic risk factors in terms of

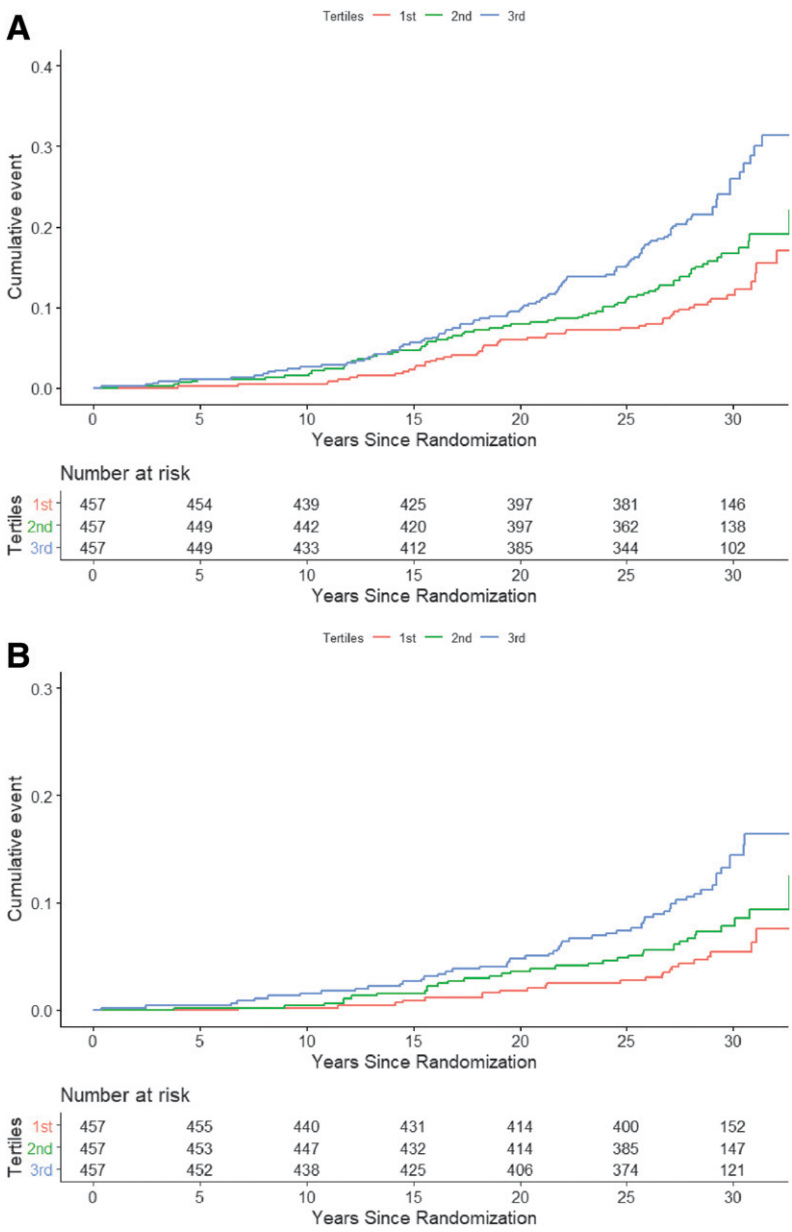

Figure 1—Cumulative incidence of CVD ($P < 0.0001$) (*A*) and MACE ($P = 0.0005$) (*B*) differed by CAD PRS tertile.

prediction improvement when added to the clinical risk factors was evaluated using the area under the receiver operating characteristic curve in models adjusted for the baseline values of those clinical factors (18). AUC describes the predictive accuracy of a model, with an AUC of 0.5 corresponding to chance predictions and an AUC of 1 corresponding to perfect predictions. The prediction improvement due to the genetic factor was the difference between the AUC for the model with the genetic factor and that for the model without the genetic factor, with CIs for the difference obtained using bootstrapping (with 2,000 bootstrap samples).

Data and Resource Availability

DCCT/EDIC data files are available at the National Institute of Diabetes and Digestive and Kidney Diseases Repository (https://repository.niddk.nih.gov/studies/edic/). Genetic data are available from dbGaP (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000086.v3.p1).

RESULTS

At DCCT baseline, the 1,371 participants in this study were similar to the full cohort (n = 1,441) (Supplementary Table 1). At baseline, the participants had a mean age of 27 years, 53% were male, 19% were smokers, the mean duration of diabetes was 5.8 years, and the mean HbA_{1c} value was 8.9% (73.8 mmol/mol). The baseline characteristics were similar across the CAD PRS tertiles, with the exception of higher LDLc values in the third (highest) CAD PRS tertile (Table 1).

As of 18 May 2017, among the 1,371 participants in this study, there were 227 CVD events over an average follow-up of 26.6 years (rate, 6.22 CVD events per 1,000 PYs) and 112 MACE over an average follow-up of 27.4 years (rate, 2.98 MACE events per 1,000 PYs). Supplementary Table 2 reports the number of CVD events separately by CAD PRS tertile and quartile. Cumulative incidence curves differed by CAD PRS tertile for both CVD (Fig. 1*A*) ($P < 0.0001$) and MACE (Fig. 1*B*) ($P = 0.0005$), with participants in the highest tertiles of CAD PRS experiencing a significantly higher incidence of events.

In age-adjusted analyses it was estimated that over the 30 years of follow-up participants with CAD PRS values in the highest tertile had their first CVD event 0.88 years earlier than individuals with CAD PRS values in the combined lower two tertiles ($P = 0.004$), for a restricted mean time-loss ratio of 1.60 ($P = 0.001$). For MACE, participants with CAD PRS values in the top tertile had their first MACE 0.56 years earlier than individuals with CAD PRS values in the lower two tertiles combined ($P = 0.010$), for a restricted mean time-loss ratio of 1.94 ($P = 0.002$). For comparison, over the 30 years of follow-up and adjusted for CAD PRS, participants with age at baseline in the upper tertile had their first CVD event 1.93 years earlier than individuals with age at baseline in the combined lower two tertiles ($P < 0.001$), for a restricted mean time-loss ratio of 2.65 ($P < 0.001$). For MACE, participants with age at baseline in the top tertile had their first MACE 1.14 years earlier than individuals with age at baseline in the lower two tertiles combined ($P < 0.001$), for a restricted

Table 2—Cox proportional hazards models assessing association between CAD PRS and risk of CVD and MACE

	Unadjusted			Adjusted for age and HbA_{1c}			Fully adjusted*		
	HR (95% CI)	*z* score	*P*	HR (95% CI)	*z* score	*P*	HR (95% CI)	*z* score	*P*
CVD									
Highest 33%†	1.83 (1.41, 2.37)	4.52	6.1E−6	1.80(1.38, 2.34)	4.40	1.1E−5	1.75 (1.34, 2.28)	4.10	4.1E−5
Per SD increase	1.42 (1.25, 1.62)	5.26	1.4E−7	1.42 (1.25, 1.62)	5.34	9.2E−8	1.38 (1.21, 1.58)	4.81	1.5E−6
MACE									
Highest 33%†	1.96 (1.36, 2.85)	3.57	3.6E−4	1.89 (1.31, 2.74)	3.37	7.4E−4	1.80 (1.24, 2.62)	3.07	2.1E−3
Per SD increase	1.50 (1.25, 1.80)	4.31	1.7E−5	1.47 (1.23, 1.76)	4.23	2.3E−5	1.40 (1.17, 1.67)	3.73	1.9E−4

Data are given as HRs with 95% Wald CIs. Models are unadjusted, adjusted for age and mean updated HbA_{1c}, and fully adjusted. *The fully adjusted models included the clinical risk factors identified in our previous work (4). The fully adjusted model for CVD was further adjusted for age, time-weighted HbA_{1c}, (log) triglycerides, time-weighted SBP, time-weighted pulse, family history of MI, duration of T1D, ACE inhibitor use, and time-weighted LDLc. The fully adjusted model for MACE was further adjusted for age, time-weighted HbA_{1c}, smoking, (log) triglycerides, time-weighted SBP, time-weighted pulse, duration of T1D, ACE inhibitor use, and current LDLc. †Comparison between individuals with CAD PRS values in the highest tertile (highest 33%) vs. the participants in the lower two tertiles combined.

mean time-loss ratio of 3.57 ($P < 0.001$).

Table 2 reports the association between CAD PRS and the risk of CVD and MACE in unadjusted models, models minimally adjusted for age and mean updated HbA_{1c}, and fully adjusted models. CAD PRS were evaluated by tertile and as a continuous variable. In unadjusted analyses, participants with CAD PRS values in the highest tertile had an 83% higher risk of CVD (hazard ratio [HR] 1.83) compared with participants with CAD PRS values in the lowest two tertiles. Compared with participants in the lower two tertiles, the risk of CVD among participants in the top tertile was 80% higher after adjustment for age and HbA_{1c} and 75% higher in the fully adjusted model. The association between CAD PRS and the risk of CVD was highly significant ($P < 0.0001$) in all models regardless of how CAD PRS was quantified (i.e., as a binary or a continuous variable). The associations were slightly attenuated but remained highly significant after minimal and full adjustment. Interaction terms between CAD PRS and mean updated HbA_{1c} and between GRS and mean updated HbA_{1c} in the fully adjusted models were not statistically significant for either CVD or MACE (data not shown).

The HRs for all covariates in the fully adjusted models with CAD PRS included as a continuous variable are summarized in Supplementary Table 3*A* for CVD and in Supplementary Table 3*B* for MACE. CAD PRS was the third most significant risk factor after age and mean HbA_{1c} for both CVD and MACE. Note that the Akaike IC (smaller values are better) value of 2,943.08 for the fully adjusted model for CVD without CAD PRS decreased to 2,921.67 after further adjustment for CAD PRS. Likewise, the Akaike IC of 1,432.56 for the fully adjusted MACE model without CAD PRS decreased to 1,420.55 after further adjustment for CAD PRS. A decrease in the Akaike IC value of $\sim$2 after further adjustment for a 1-df variable is typically considered important.

Changes in the HRs and *z* scores for most risk factors between the fully adjusted models with and without further adjustment for CAD PRS were minor (Supplementary Table 3*A* and *B*). The largest change was for mean weighted LDLc, which had a 33% reduction in the HR after adjustment for CAD PRS (from HR 1.0078; $z = 2.49$ to HR 1.0052; $z = 1.62$). To investigate this further, we also report fully adjusted models that include CAD PRS but do not include time-weighted LDLc (CVD, Supplementary Table 3*A*; MACE, Supplementary Table 3*B*). For CVD, adjustment for mean weighted LDLc only resulted in a 6.4% reduction in the HR for CAD PRS (Supplementary Table 3*A*), while for MACE, adjustment for mean weighted LDLc only resulted in a 6.5% reduction in the HR for CAD PRS (Supplementary Table 3*B*). Note that models without mean weighted LDLc but with CAD PRS had much lower Akaike ICs (and therefore are better) for both CVD and MACE than models with mean weighted LDLc but without CAD PRS. A GEE longitudinal model showed that CAD PRS values were strongly associated with time-weighted LDLc values ($P = 1.2E-9$), independent of age, time-weighted HbA_{1c}, duration of T1D, and use of lipid-lowering medication (Supplementary Table 4 and Supplementary Fig. 2).

Supplementary Table 5 describes the associations between the weighted and unweighted GRS and the risk of CVD and MACE in unadjusted models, models minimally adjusted for age and mean updated HbA_{1c}, and fully adjusted models with and without further adjustment for CAD PRS. Both weighted and unweighted CAD GRS were associated with CVD and MACE in the unadjusted and the minimally adjusted models. The weighted GRS, but not the unweighted GRS, remained significantly associated with the risk of outcomes in the fully adjusted models before further adjustment for CAD PRS. Neither the weighted nor unweighted GRS remained significant in the fully adjusted models after further adjustment for CAD PRS.

Several individual SNPs were significantly associated with the risk of CVD and MACE in separate fully adjusted models (Supplementary Table 6). Table 3 reports multivariable models for CVD and MACE that included the CAD PRS and all individual SNPs significantly associated with either of the two outcomes. For CVD, CAD PRS was the fourth most significant risk factor ($z = 3.44$) after age ($z = 7.6$), mean updated HbA_{1c} ($z = 4.9$), and (log) triglycerides ($z = 4.4$). Individual SNPs significantly associated with the risk of CVD were chr4_148401190_G_A (rs6841581), chr12_118265441_T_G (rs11830157), and

Table 3—Association of clinical risk and genetic factors with subsequent risk of CVD and MACE in separate multivariable Cox proportional hazards models

Covariate	HR (95% CI)	*z* score	*P*
CVD (Akaike IC 2,909.790)			
Age	1.09 (1.06, 1.11)	7.6014	**2.9E−14**
Mean updated HbA_{1c}	1.39 (1.22, 1.58)	4.9452	**7.6E−7**
Log triglycerides	1.75 (1.37, 2.25)	4.4327	**9.3E−6**
CAD PRS	1.34 (1.18, 1.53)	4.3637	**1.3E−5**
Mean updated SBP	1.03 (1.01, 1.05)	3.3602	**7.8E−4**
Mean updated pulse	1.02 (1.00, 1.05)	2.2041	**2.8E−2**
Mean updated LDLc	1.01 (1.00, 1.01)	1.9127	5.6E−2
Duration of T1D	1.00 (1.00, 1.00)	1.7542	7.9E−2
Family history of MI	1.26 (0.96, 1.66)	1.6843	9.2E−2
Any ACE inhibitor use	0.78 (0.58, 1.05)	1.6373	1.0E−1
chr4_148401190_G_A (2 vs. 1) (rs6841581)*	2.07 (0.94, 4.57)	1.8066	7.1E−2
chr4_148401190_G_A (1 vs. 0) (rs6841581)*	1.35 (1.01, 1.80)	2.0338	**4.2E−2**
chr12_118265441_T_G (2 vs. 1) (rs11830157)	1.55 (1.09, 2.22)	2.4147	**1.6E−2**
chr12_118265441_T_G (1 vs. 0) (rs11830157)	0.69 (0.51, 0.92)	2.5053	**1.2E−2**
chr19_33386556_T_C (2 vs. 1) (rs10417115)	7.59 (2.23, 25.77)	3.2487	**1.2E−3**
chr19_33386556_T_C (1 vs. 0) (rs10417115)	1.07 (0.74, 1.56)	0.3656	7.1E−1
MACE (Akaike IC 1,407.239)			
Age	1.10 (1.07, 1.14)	6.3796	**1.8E−10**
Mean updated HbA_{1c}	1.52 (1.27, 1.83)	4.5778	**4.7E−6**
CAD PRS	1.39 (1.16, 1.67)	3.5899	**3.3E−4**
Log triglycerides	1.74 (1.21, 2.49)	3.0138	**2.6E−3**
Current smoker	1.92 (1.25, 2.95)	2.9642	**3.0E−3**
Duration of T1D	1.01 (1.00, 1.01)	2.6169	**8.9E−3**
Mean updated SBP	1.03 (1.00, 1.05)	2.3269	**2.0E−2**
LDLc	1.01 (1.00, 1.01)	2.2803	**2.3E−2**
Mean updated pulse	1.03 (1.00, 1.06)	1.7214	8.5E−2
Any ACE inhibitor use	0.72 (0.48, 1.09)	1.5500	1.2E−1
chr4_148401190_G_A (2 vs. 1) (rs6841581)	2.86 (1.08, 7.55)	2.1185	**3.4E−2**
chr4_148401190_G_A (1 vs. 0) (rs6841581)	1.17 (0.76, 1.79)	0.7245	4.7E−1
chr6_31888367_C_T (2 vs. 1) (rs3130683)	0.37 (0.21, 0.63)	3.5833	**3.4E−4**
chr6_31888367_C_T (1 vs. 0) (rs3130683)†	n/a		
chr18_57832856_A_AC (2 vs. 1) (rs35614134)	0.61 (0.24, 1.54)	1.0443	3.0E−1
chr18_57832856_A_AC (1 vs. 0) (rs35614134)	1.77 (1.20, 2.60)	2.9001	**3.7E−3**
chr19_33386556_T_C (2 vs. 1) (rs10417115)	8.70 (1.88, 40.32)	2.7663	**5.7E−3**
chr19_33386556_T_C (1 vs. 0) (rs10417115)	1.00 (0.58, 1.72)	0.0045	9.9E−1

Boldface type indicates significance at $P < 0.05$. The multivariable models include the clinical risk factors identified in our previous work (4) (see footnote in Table 2) and the genetic risk factors that were individually significantly associated with the risk of CVD and MACE. n/a, not applicable. *Genetic variable label is CHR_BP_A1_A2 (rs identifier), in which A2 is the effect allele. †No HR is listed due to a lack of events for this allele count.

chr19_33386556_T_C (rs10417115). For MACE, CAD PRS was the third most significant factor (z = 3.6) after age (z = 6.4) and mean updated HbA_{1c} (z = 4.6). Individual SNPs significantly associated with the risk of MACE were chr4_148401190_G_A (rs6841581), chr6_31888367_C_T (rs3130683), chr18_57832856_A_AC (rs35614134), and chr19_33386556_T_C (rs10417115).

The fully adjusted baseline clinical model for CVD had an AUC of 0.698. Adding CAD PRS significantly increased the AUC for CVD to 0.712 (for a difference of 0.014; 95% CI 0.002, 0.034 for the difference). Adjustment for the weighted GRS also marginally increased the AUC for CVD to 0.703 (for a difference of 0.002; 95% CI 5.04E−05, 0.019 for the difference). The fully adjusted baseline clinical model for MACE had an AUC of 0.720, which increased to 0.734 (for a difference of 0.014; 95% CI −5E−4, 0.038 for the difference) after adjustment for CAD PRS and to 0.728 (for a difference of 0.008; 95% CI −1E−4, 0.030 for the difference) after adjustment for the weighted GRS. Adjustment for the unweighted GRS did not result in a significant increase of the AUC for either CVD or MACE (data not shown).

CONCLUSIONS

In this study, we evaluated the effect of a CAD PRS based on ∼6.6 million SNPs (5) as a risk factor for CVD and MACE in participants with T1D in the DCCT/EDIC. We compared these effects with two other CAD GRS (unweighted and weighted CAD GRS) using 78 SNPs (12). The genetic risk factors for CAD considered in our analyses were obtained from prior independent analyses of other cohorts predominantly without diabetes. Most were cross-sectional studies. Our prospective analyses independently extend these associations to the risk of incident CVD, including other cardiovascular events beyond CAD, in people with T1D. Moreover, the associations of CAD PRS with CVD remained highly significant even after adjustment for established and putative clinical CVD risk factors measured over time. In particular, when evaluated as a quantitative

risk factor, CAD PRS was the third strongest risk factor (after age and glycemia) for both CVD and MACE.

Note that the HRs were higher for the MACE models compared to the CVD models (Table 2). However, owing to the smaller number of events (n = 112 MACE events vs. n = 227 CVD events), there was less power, and the z scores were smaller (and equivalently, the P values were larger) in the MACE models compared with the CVD models. Of note, MACE in the DCCT/EDIC cohort more closely resembles the clinical events on which the CAD PRS was based.

While CAD PRS was associated with LDLc, especially during the DCCT time period (Supplementary Fig. 2), CAD PRS was more strongly associated with CVD risk than LDLc. There was little change in the associations of the other clinical factors and the risk of CVD after adjustment for CAD PRS, suggesting that CAD PRS represents different causal pathways not captured by these clinical risk factors.

It is important to note that the order in which the genetic factors were tested was prespecified, namely CAD PRS first, followed by the weighted and unweighted GRS and then by the individual SNPs. Therefore, no adjustment for multiplicity was required for the CAD PRS analyses. Given the exploratory nature of our analyses with regard to the individual SNPs, no adjustment for multiplicity was conducted for the SNPs. However, none of the observed associations for the individual SNPs (Supplementary Table 6) remained significant after multiplicity adjustment for the 78 tests.

PRS were positively associated with the risk of incident CAD events in the general population (19–21), although the associations were much weaker in individuals with prevalent CAD events (20). A positive association between GRS levels and the risk of subsequent CVD events was also shown in T2D in the ACCORD study (22).

However, it is still unclear whether the polygenic risk factors can improve prediction and prevention of CAD events. Previous studies have shown mixed but mostly negative results in the general population (5,19,23,24) and some minor improvement in T2D in the ACCORD study (22). In our T1D cohort, when added to a model adjusted for known clinical risk factors, the CAD PRS resulted in a small but significant improvement of the AUC, a measure of predictive ability, for CVD from 0.698 to 0.712. A similar improvement was observed in the AUC for MACE (from 0.720 to 0.734), but the difference was not statistically significant, likely due to the smaller number of MACE (n = 112) compared with CVD events (n = 227). While these results are encouraging, more research is needed before these PRS can be used in clinical practice.

The major strength of our study is the availability of standardized measurements of established and putative risk factors over an extended period of time and of independently adjudicated cardiovascular outcomes assessed prospectively over approximately three decades, with 94% of the surviving cohort still actively participating in the study. Limitations of our study include the exclusion at baseline of individuals with CAD, hyperlipidemia, or hypertension and some individuals with albuminuria. Moreover, the DCCT/EDIC cohort is mostly White, consistent with the ethnic distribution of T1D; however, our findings need to be validated in different race/ethnicity groups. It should also be noted that the PRS and individual genetic variants considered in our analyses were initially identified for CAD and not for MACE or CVD, which were examined in this report. While this difference may, therefore, underestimate the association of CAD PRS with the risk of events, it also extends the potential impact of the CAD PRS. An analysis using a composite CAD outcome defined as acute/silent MI and/or percutaneous transluminal coronary angioplasty/coronary artery bypass grafting (n = 179) yielded results similar to those for CVD and MACE (Supplementary Table 7).

In summary, our results support the role of genetic factors in the subsequent risk of CVD in individuals with T1D, above and beyond the effect of established risk factors such as age, glycemia, lipids, and BP. Of note, only age and chronic glycemia are more potent risk factors than the PRS in the DCCT/EDIC cohort.

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