Genetic Predictors of Cardiovascular Mortality During Intensive Glycemic Control in Type 2 Diabetes: Findings From the ACCORD Clinical Trial

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OBJECTIVE

To identify genetic determinants of increased cardiovascular mortality among subjects with type 2 diabetes who underwent intensive glycemic therapy in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial.

RESEARCH DESIGN AND METHODS

A total of 6.8 million common variants were analyzed for genome-wide association with cardiovascular mortality among 2,667 self-reported white subjects in the ACCORD intensive treatment arm. Significant loci were examined in the entire ACCORD white genetic dataset (n = 5,360) for their modulation of cardiovascular responses to glycemic treatment assignment and in a Joslin Clinic cohort (n = 422) for their interaction with long-term glycemic control on cardiovascular mortality.

RESULTS

Two loci, at 10q26 and 5q13, attained genome-wide significance as determinants of cardiovascular mortality in the ACCORD intensive arm ($P = 9.8 \times 10^{-9}$ and $P = 2 \times 10^{-8}$, respectively). A genetic risk score (GRS) defined by the two variants was a significant modulator of cardiovascular mortality response to treatment assignment in the entire ACCORD white genetic dataset. Participants with GRS = 0 experienced a fourfold reduction in cardiovascular mortality in response to intensive treatment (hazard ratio [HR] 0.24 [95% CI 0.07–0.86]), those with GRS = 1 experienced no difference (HR 0.92 [95% CI 0.54–1.56]), and those with GRS ≥2 experienced a threefold increase (HR 3.08 [95% CI 1.82–5.21]). The modulatory effect of the GRS on the association between glycemic control and cardiovascular mortality was confirmed in the Joslin cohort (P = 0.029).

CONCLUSIONS

Two genetic variants predict the cardiovascular effects of intensive glycemic control in ACCORD. Further studies are warranted to determine whether these findings can be translated into new strategies to prevent cardiovascular complications of diabetes.

As diabetes becomes a worldwide epidemic, there is a critical need to enhance prevention of its cardiovascular complications as these are responsible for a large part of the increased morbidity, mortality, and socioeconomic burden of this disease (1–3). Since hyperglycemia is the defining characteristic of diabetes, near normalization of blood glucose levels by intensive glycemic control has been proposed as one of the interventions that can be used for this purpose. A meta-analysis of four large randomized clinical trials in subjects with type 2 diabetes has indeed shown that this intervention can lower the risk of myocardial infarction by 15% and that of major cardiovascular events by 9% (4). However, in one of these studies, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, intensive glycemic control was associated with a paradoxical increase in mortality, mainly due to increased cardiovascular deaths (5). This prompted early termination of the trial's intensive arm 3.7 years postrandomization. Results of an intention-to-treat analysis at 5 years from randomization showed an 18% significant risk reduction in nonfatal myocardial infarctions, which, however, was offset by a 29% significant increase in cardiovascular mortality (6). While the reasons for this paradoxical rise in mortality are being debated (7–11), we sought to identify genetic predictors of this adverse effect of intensive glycemic control that could be used to select individuals with type 2 diabetes who could be safely treated with this intervention. To this end, we conducted a genome-wide association study (GWAS) of cardiovascular mortality in the ACCORD intensive arm and analyzed the modulating influence of significant loci on the effects of intensive and standard treatments on fatal and nonfatal cardiovascular outcomes. These loci were further investigated in a cohort of patients with type 2 diabetes from the Joslin Clinic as well as in the Outcome Reduction With Initial Glargine Intervention (ORIGIN) trial cohort.

RESEARCH DESIGN AND METHODS

Study Cohorts

ACCORD Study

ACCORD was designed to test the effect of intensive glycemic control (targeting glycated hemoglobin [HbA_{1c}] levels to $<$6.0% [42 mmol/mol]) on cardiovascular outcomes in type 2 diabetes, as compared with a standard therapy aimed at HbA_{1c} levels of 7–7.9% (53–63 mmol/mol) (5). The study included 10,251 participants with type 2 diabetes and high cardiovascular risk from the U.S. and Canada. Subjects were randomized in a 1:1 ratio to intensive and standard glycemic arms as well as to blood pressure and lipid subtrials in a double 2 $\times$ 2 factorial design (5). Detailed rationale, methods, and results of the trial have been published previously (12). DNA samples from 8,174 ACCORD participants (79.7% of 10,251), who had consented for genetic studies, were assayed by genome-wide genotyping. After application of the genotyping quality control (QC) procedures described in the Supplementary Data (Supplementary Material 1, Supplementary Figs. 1–5, and Supplementary Tables 1–3), 8,084 samples remained. Baseline characteristics and distribution between treatment arms of these 8,084 subjects were similar to those of subjects who were not included in the genetic study, with few exceptions (Supplementary Table 4). The effects of intensive glycemic control on the risk of cardiovascular death and nonfatal myocardial infarction were similar to those reported in the entire ACCORD study (hazard ratio [HR] 1.47 [95% CI 1.12–1.93] and HR 0.81 [95% CI 0.68–0.97], respectively).

Joslin Kidney Study in Type 2 Diabetes

Significant single nucleotide polymorphisms (SNPs) identified from the ACCORD GWAS were examined in a cohort of subjects with type 2 diabetes from the Joslin Kidney Study in Type 2 Diabetes (JKS). This cohort was a random sample (n = 516) of Joslin Clinic patients enriched for micro- and macroalbuminuria that were recruited between 1993 and 1996 (13). Our study was limited to 422 study participants who were self-reported whites and for whom DNA samples were still available in 2015.

ORIGIN Study

Significant SNPs were further investigated in the ORIGIN trial (NCT00069784), design and results of which have been previously published (14). In brief, 12,537 individuals with dysglycemia and additional cardiovascular risk factors were followed for a median of 6.2 years for development of cardiovascular outcomes. Participants were allocated to insulin-mediated normoglycemia using glargine insulin versus standard care and n-3 fatty acids versus placebo using a 2 $\times$ 2 factorial design. The current study was conducted in 1,931 white participants for whom DNA samples were available. These individuals suffered 167 cardiovascular deaths during up to 7 years of follow-up. Replication was not sought in any study other than JKS or ORIGIN.

DNA Extraction and Genotyping

ACCORD Study

Genomic DNA was extracted from white cells at the University of Washington using the FlexiGene DNA Kit (Qiagen, Valencia, CA) (15). Genome-wide genotyping was performed in two independent laboratories on different platforms: 6,085 samples, corresponding to those ACCORD participants who had consented to genetic studies conducted by any investigator, were genotyped at the University of Virginia (UVA) on Illumina HumanOmniExpressExome-8 v1.0 chips; and 8,174 samples, including the above 6,085 samples plus 2,089 samples from ACCORD participants who had consented to genetic studies only if conducted by ACCORD investigators, were genotyped at the University of North Carolina (UNC) on Affymetrix Axiom Biobank1 chips. After extensive within-laboratory QC, the data were merged with further between-laboratory QC, resulting in two nonoverlapping sets of samples: ANYSET, including 5,971 samples genotyped at either UVA or UNC at a total of 1,263,585 individual SNPs, and ACCSET, including 2,113 samples genotyped only at UNC at 572,192 SNPs. The two sets were imputed to over 24 million high quality SNPs using Impute v2.3.1 (16). Additional details about the genotyping, QC, and merge procedures are provided in the Supplementary Data (Supplementary Material 1, Supplementary Figs. 1–5, and Supplementary Tables 1–3).

JKS

Significant variants from the ACCORD GWAS were genotyped by the Joslin Advanced Genomics and Genetics Core by means of custom TaqMan assays (Life Technologies, Foster City, CA). Genotyping quality was tested by including six blinded duplicate samples in each 384-well assay. The average agreement rate of duplicate samples was $>$99%.

ORIGIN Study

Genotyping was done using the HumanCoreExome Beadchip-12 v1.0 and v1.1 (Illumina) that measured 551,839 markers, which also allowed the imputation of ~30 million SNPs using Impute v2.3.1. The rs57922 SNP was in the imputed data and the rs9299870 was not available, but a proxy in complete linkage disequilibrium (rs1762431; $r^2 = 1$) was used in place of this SNP.

Outcomes

ACCORD Study

Cardiovascular mortality, as previously defined by the ACCORD study group (12), encompassed all deaths due to myocardial infarction, congestive heart failure, arrhythmia, stroke, invasive cardiovascular interventions, unexpected deaths due to ischemia occurring within 24 h after symptom onset, and other vascular causes of death. Nonfatal myocardial infarction was diagnosed by the presence of cardiac enzyme elevation and new significant Q waves on electrocardiography (12).

JKS

Deaths as of December 2011 were determined by matching with the National Death Index (13). A death was attributed to cardiovascular causes if the primary cause of death was coded as ICD-9 codes 401–448.9 or ICD-10 codes I10–I74.9, or if cardiovascular disease was listed as the secondary cause of death and diabetes or renal failure listed as the primary cause (13).

ORIGIN

A cardiovascular cause of death was presumed if no definite noncardiovascular causes were identified (14). This included sudden unexpected deaths, unwitnessed deaths, and deaths due to arrhythmia, myocardial infarctions, heart failure, invasive cardiovascular interventions, stroke, other vascular events, and unknown causes (14).

Data Analysis

ACCORD Study

The primary goal of the study was to identify associations between common genetic variants (minor allele frequency [MAF] $\geq$0.05) and cardiovascular mortality in the intensive treatment arm. To avoid possible confounding and/or heterogeneity in linkage disequilibrium patterns due to racial differences, the analysis was restricted to self-reported non-Hispanic white subjects in this arm (n = 2,667).

Due to the differences in genotyping platforms, independent genome-wide analyses were performed in the two genotyping sets (including 2,145 and 522 individuals in the ANYSET and ACCSET, respectively) and results meta-analyzed. For each variant, the expected minor allele dosage, ranging from 0 to 2, was computed from the imputed posterior genotype probabilities. Subsequent statistical analyses were conducted using SAS v9.4 (SAS Institute, Cary, NC). The association between minor allele dosage and cardiovascular mortality was evaluated for each variant by means of Cox proportional hazards regression assuming an additive genetic model. As in the original ACCORD analysis (5,6), the regression models included indicators for the seven clinical center networks, blood pressure or lipid subtrials assignment, and treatment assignments within these subtrials as covariates, along with adjustments for the first three principal components, PC1–PC3, which explain a large part of the population admixture of the ACCORD cohort (Supplementary Fig. 4). All cardiovascular deaths observed in the intensive arm until the end of the study in self-reported non-Hispanic whites (n = 84) were included in the analysis. After filtering the results by MAF $\geq$0.05 and applying a genomic control correction (λ = 1.02 and 0.92 for ANYSET and ACCSET, respectively), results from the two genotyping sets were summarized by means of a fixed-effects meta-analysis using an inverse-variance approach in METAL (17). Meta-analysis results were considered significant if the P value for the variant was less than the genome-wide significance threshold of 5×10^{-8} and notable (suggestive) if $<1 \times 10^{-6}$. Further analyses of variants showing significant or notable associations were conducted among self-reported whites to estimate their effects in the standard therapy group, test for their interaction with treatment assignment, and investigate the effect of a genetic risk score (GRS) calculated by adding the minor allele dosage at the two genome-wide significant loci. The effect of significant variants on nonfatal myocardial infarction was explored in a similar fashion. Kaplan-Meier plots were generated to illustrate the effect of significant variants and to estimate the number of cardiovascular events caused or prevented by treating 1,000 subjects with intensive as opposed to standard therapy for 5 years (18).

The top two variants were further examined in the Genotype Tissue Expression (GTEx) database (http://www.gtexportal.org/home/) (19) for their correlation with tissue-specific gene expression levels. Genes within 1Mb from either SNP were selected and only tissues that had at least 70 donor samples in the database with matched gene expression and genotype data were included in the analysis. For each tissue and gene, the effect of the SNP minor allele on gene expression was analyzed by linear regression. Beta estimates were then meta-analyzed across all tissues by generic inverse-variance methods.

JKS

The average degree of glycemic control while attending the Joslin Clinic was estimated at baseline and at the end of each year of follow-up as the time-weighted average of all HbA_{1c} measurements available at the Joslin from 1990 (inception of electronic Joslin laboratory records) up to that point in time. These yearly HbA_{1c} averages were used to build a cumulative, time-dependent index of glycemic control. For measurements taken before 1994, HbA_{1c} values were derived from HbA_{1c} values as previously described (20). The interaction between good glycemic control (defined as a time-dependent mean HbA_{1c} in the lowest quartile [<7.5% (58 mmol/mol)]) and GRS (constructed from the two lead SNPs of the ACCORD GWAS) on cardiovascular mortality was evaluated by means of Cox proportional hazards regression adjusting for age and sex. The JKS first quartile (7.5%) corresponds to the 87th and 43rd percentiles of mean follow-up HbA_{1c} in the ACCORD intensive and standard arms, respectively. Thus, this cutoff was a good index within the JKS to reproduce the contrast between intensive and standard control while providing adequate power. The time variable was defined as the time between study entry and the date of death, or, for subjects who did not die, the date of censoring (31 December 2011).

ORIGIN Study

The association between GRS (constructed from the two lead SNPs of ACCORD) and

cardiovascular mortality was analyzed in ORIGIN by means of Cox proportional hazards regression adjusting for age, sex, and treatment assignment. Additional analyses were performed to determine whether there was an interaction between GRS and glargine allocation or achieved HbA_{1c} (considered as a time-varying covariate) on cardiovascular mortality.

RESULTS

Genome-Wide Association Analysis

After filtering by MAF ≥0.05, a total of 6,839,462 high-quality common variants were analyzed for association with cardiovascular mortality among 2,667 self-reported white subjects from the ACCORD intensive therapy arm. Baseline characteristics of these subjects did not differ from individuals in the standard glycemic arm (Supplementary Table 5). Manhattan and quantile-quantile plots summarize the results (Fig. 1). Two loci reached genome-wide significance ($P < 5 \times 10^{-8}$). One was placed on chromosome 10, within intron 1 of the *MGMT* (O-6-methylguanine-DNA methyltransferase) gene (Supplementary Fig. 6). The lead SNP at this location (rs9299870) had a MAF of 0.08 and was associated with a 3.6-fold increased risk of cardiovascular death per minor allele copy ($P = 9.8 \times 10^{-9}$) (Table 1). The other locus was placed on chromosome 5, upstream and proximal to three long intergenic noncoding (LINC) RNAs (*LINC1335*, *LINC1333*, and *LINC1331*) (Supplementary Fig. 7). The lead SNP at this location (rs57922) had a MAF of 0.48 and was associated with a 2.7-fold increased risk of cardiovascular death per minor allele copy ($P = 2 \times 10^{-8}$) (Table 1). The two lead SNPs were well-imputed variants in both ANYSET and ACCSET (Supplementary Table 6). There were also close-by genotyped markers in strong linkage disequilibrium that supported these associations (rs569120 at 5q13 [$P = 3.8 \times 10^{-8}$] and rs76496923 at 10q26 [$P = 2.6 \times 10^{-7}$]). Both lead associations were unaffected by adjustment for history of cardiovascular disease at baseline, age, and sex (Supplementary Table 7). Neither locus was associated with noncardiovascular mortality (HR 1.00 [95% CI −0.59 to 1.69] and HR 0.86 [95% CI 0.65–1.13], respectively).

Three other loci, placed on chromosomes 1, 11, and 5, showed notable yet non–genome-wide significant associations with cardiovascular mortality (P values in the 1×10^{-6} to 5×10^{-8} range). The locus on chromosome 1 reached genome-wide significance after adjustment for baseline history of cardiovascular disease ($P = 3.6 \times 10^{-8}$) and further adjustment for age at baseline and sex ($P = 2.4. \times 10^{-8}$). Nineteen other loci were associated with P values in the 1×10^{-5} to 1×10^{-6} range (Table 1).

Interaction Between Genetic Variants and Intensive Glycemic Treatment

The two loci with genome-wide significance in the intensive arm of ACCORD were not associated with cardiovascular mortality in the standard treatment arm (HR 0.96 and $P = 0.91$ for rs9299870, and HR 1.07 and $P = 0.72$ for rs57922) (Table 1). This translated into gene × treatment interaction P values of 0.004 and 0.0004, respectively; although these P values were likely biased downward by selecting SNPs with extreme association P values in the intensive glycemic control arm for interaction analysis. These interactions are illustrated in Fig. 2 as the influence of the two loci on the effect of intensive therapy on cardiovascular mortality compared with standard treatment. Allocation to intensive treatment led to a threefold increase in cardiovascular mortality among rs9299870 minor allele carriers (HR 2.96 [95% CI 1.38–6.36]), whereas it had no detrimental effect on this outcome among major allele homozygotes (HR 1.14 [95% CI 0.78–1.67]) (Fig. 2*A*). Similarly, intensive treatment led to a 2.8-fold increase in cardiovascular mortality among rs57922 minor allele homozygotes (HR 2.83 [95% 1.56–5.15]) but had no significant effect among major

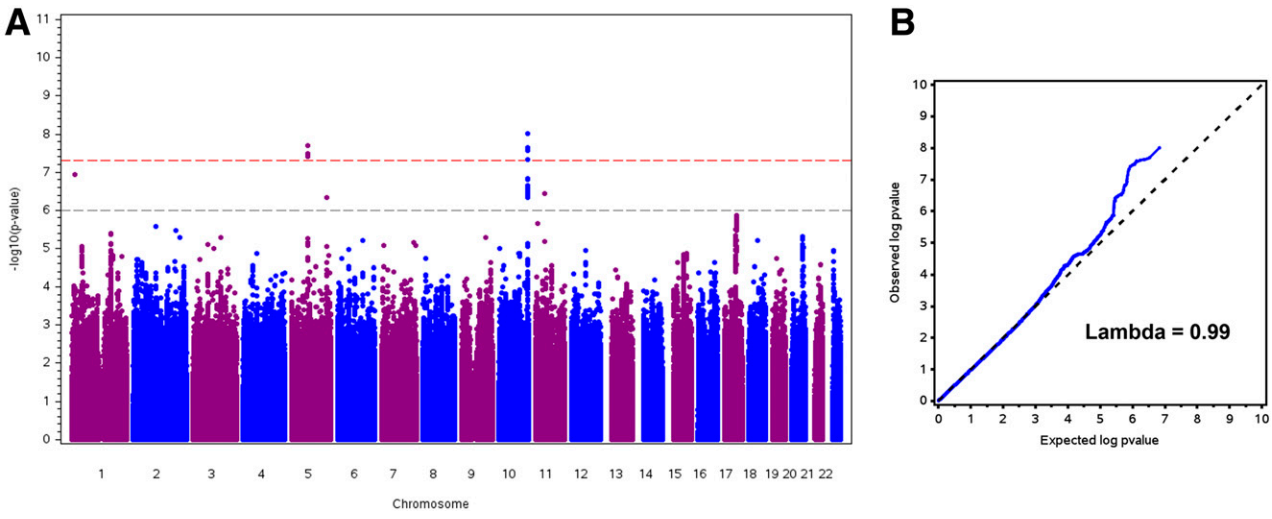

Figure 1—Genome-wide association results. *A*: The genomic distribution of P values (Manhattan plot) for association with time to cardiovascular mortality at 6.8 million common polymorphic loci in 2,667 self-reported whites from the ACCORD intensive glycemic treatment arm. P values are plotted as $-\log_{10}$ values to facilitate visualization. Each dot represents a polymorphism. The top dashed reference line corresponds to the genome-wide significance threshold ($P = 5 \times 10^{-8}$), whereas the lower dashed line corresponds to the notable significance level ($P = 1 \times 10^{-6}$). *B*: The relationship between observed and expected P values (quantile-quantile, or Q-Q plot) in the genome-wide analysis. The dotted line corresponds to the null hypothesis; lambda is the genomic inflation factor.

Table 1—Top GWAS loci ($P < 1 \times 10^{-5}$) associated with cardiovascular mortality: effects in the intensive and standard glycemic treatment arms

				Intensive arm (n = 2,667)		Standard arm (n = 2,693)		Overall
Closest gene*	SNP†	Position‡	MAF§	HR (95% CI)	P	HR (95% CI)	P	P for interaction¶
MGMT	rs9299870	10:131269309	0.08	3.58 (2.32–5.55)	9.77E-09	0.96 (0.48–1.92)	0.91	0.0042
LINC01333	rs57922	5:73577939	0.48	2.65 (1.88–3.72)	2.04E-08	1.07 (0.75–1.53)	0.72	0.0004
MASP2	rs373946618	1:11088774	0.08	4.00 (2.40–6.68)	1.15E-07	0.86 (0.33–2.24)	0.75	0.0026
AX748080	rs79525442	11:43990932	0.06	2.98 (1.96–4.54)	3.63E-07	1.62 (0.84–3.13)	0.15	0.0897
CCNJL	rs6878970	5:159771753	0.06	3.10 (2.00–4.81)	4.68E-07	1.31 (0.66–2.59)	0.44	0.0399
ANKFN1	rs116899003	17:54448567	0.05	2.86 (1.87–4.39)	1.35E-06	1.34 (0.68–2.64)	0.41	0.0777
GALNT18	rs1487122	11:11472617	0.06	2.98 (1.90–4.69)	2.19E-06	2.10 (1.15–3.85)	0.02	0.3206
LINC01102	rs200457531	2:104694510	0.21	2.27 (1.61–3.20)	2.63E-06	1.03 (0.64–1.66)	0.90	0.0064
KIF2B	rs79761505	17:51588871	0.06	2.67 (1.77–4.03)	3.09E-06	1.56 (0.82–2.97)	0.17	0.1779
PCGEM1	rs200184681	2:194259469	0.05	3.29 (1.99–5.43)	3.31E-06	1.09 (0.38–3.13)	0.87	0.0575
RASAL2	rs2209169	1:178601492	0.42	2.09 (1.53–2.86)	4.07E-06	1.36 (0.95–1.96)	0.09	0.0949
TMEM189	rs55757919	20:48748548	0.21	2.13 (1.54–2.95)	4.79E-06	0.91 (0.58–1.42)	0.67	0.0017
ACTL7B	rs142631117	9:111614117	0.07	2.63 (1.73–3.98)	5.13E-06	1.09 (0.53–2.26)	0.81	0.0508
IKZF2	rs56175857	2:213929465	0.10	2.58 (1.72–3.88)	5.25E-06	1.02 (0.53–1.96)	0.95	0.0171
MIR548I1	rs140432795	3:125518739	0.05	3.29 (1.97–5.48)	5.25E-06	0.75 (0.27–2.05)	0.57	0.0117
MIR_584	rs72947763	6:115041783	0.06	2.99 (1.86–4.82)	6.17E-06	1.20 (0.54–2.64)	0.65	0.0456
SETBP1	rs56161428	18:42524278	0.06	2.76 (1.78–4.29)	6.31E-06	0.83 (0.37–1.89)	0.66	0.0163
LOC155060	rs6974847	7:148998960	0.25	2.09 (1.52–2.88)	6.92E-06	1.10 (0.73–1.66)	0.64	0.0124
SLC25A26	rs78974441	3:66343805	0.09	2.63 (1.72–4.02)	7.94E-06	1.32 (0.78–2.22)	0.30	0.0432
CNPY1	rs55907517	7:155302020	0.07	2.77 (1.77–4.33)	8.32E-06	0.25 (0.06–1.01)	0.05	0.0040
PER4	rs111891616	7:9437462	0.08	2.67 (1.73–4.11)	8.51E-06	1.45 (0.75–2.81)	0.27	0.1971
ERMAP	rs12406643	1:43311563	0.18	2.14 (1.53–2.99)	9.12E-06	0.93 (0.57–1.50)	0.76	0.0103
SUMO1P1	rs62206653	20:52538079	0.06	3.10 (1.88–5.12)	9.33E-06	1.41 (0.68–2.94)	0.36	0.1021
PFKP	rs58751041	10:3007494	0.16	2.19 (1.55–3.10)	9.77E-06	0.77 (0.43–1.38)	0.38	0.0018

Primary analysis includes adjustment for assignment to blood pressure and lipid subtrials, interventions within these subtrials, seven clinical center networks, and top three principal components. These are results of meta-analysis of the ANYSET and ACCSET; results within individual sets are shown in Supplementary Table 5. Other adjusted analyses are shown in Supplementary Table 6. *Closest gene within 500 kbp of the SNP. †One representative per locus. ‡Position is chromosome:bp. Position according to the National Center for Biotechnology Information assembly build GRCh37/hg19. §Here MAF is the average of the minor allele frequencies of ANYSET and ACCSET. ¶Effect of SNP × treatment interaction.

allele homozygotes or heterozygotes (HR 0.38 [95% CI 0.14–1.05] and HR 1.26 [95% CI 0.79–2.00], respectively) (Fig. 2*B*). Differences among rs9299870 and rs57922 genotypes also appeared to be present with regard to nonfatal myocardial infarction, with the benefit of intensive glycemic control on this outcome showing a tendency to be more evident among carriers of those genotypes that were protected from the detrimental effect on cardiovascular mortality (Fig. 2*C* and *D*). However, the evidence for a SNP × treatment interaction on this outcome did not achieve significance at either locus ($P = 0.23$ and $P = 0.24$, respectively).

An additional genome-wide screen for variants interacting with treatment assignment without being significantly associated with cardiovascular mortality in the intensive treatment arm did not yield genome-wide significant results (Supplementary Fig. 8).

Association Between Genetic Variants and Gene Expression

In the GTEx database (19), carriers of the minor allele of the lead SNP rs9299870 showed higher expression of the *MGMT* gene ($P < 0.01$) in tissues such as pancreas, spleen, aorta, and subcutaneous adipose tissue. A meta-analysis of all the 44 tissues available in GTEx yielded a P value of 4×10^{-17} ($I^2 = 0\%$) for association between rs9299870 and *MGMT* expression (Supplementary Fig. 10). In a similar meta-analysis of all tissues in the GTEx database, the top variant at 5q13 (rs57922) was associated with expression of the Nop-7–associated 2 (*NSA2)* gene located 500 kb upstream of this SNP ($P = 2 \times 10^{-11}$; $I^2 = 17\%$) (Supplementary Fig. 11).

GRS for Excess Cardiovascular Mortality in the Intensive Arm

A quantitative GRS, capturing the joint effect of rs57922 and rs9299870, was calculated by adding the minor allele dosage of the two SNPs. Individuals were subdivided into three GRS classes (0, 1, and ≥2) based on the distribution shown in Supplementary Fig. 9. Baseline characteristics of trial participants did not differ between the three GRS strata (Supplementary Table 8). Among ACCORD participants with GRS = 0 (22.6% of study participants), assignment to intensive therapy was associated with a fourfold reduction in cardiovascular mortality (HR 0.24 [95% CI 0.07–0.86]) and twofold reduction in nonfatal myocardial infarction (HR 0.56 [95% CI 0.35–0.90]) (Fig. 3*A*). Among participants with GRS = 1 (47.7% of participants), assignment to intensive glycemic control did not have any significant effect on cardiac mortality (HR 0.92 [95% CI 0.54–1.56]) while causing a 30% reduction in the risk of nonfatal myocardial infarctions (HR 0.70 [95% CI 0.52–0.94]). Among subjects with GRS ≥2 (29.6% of participants),

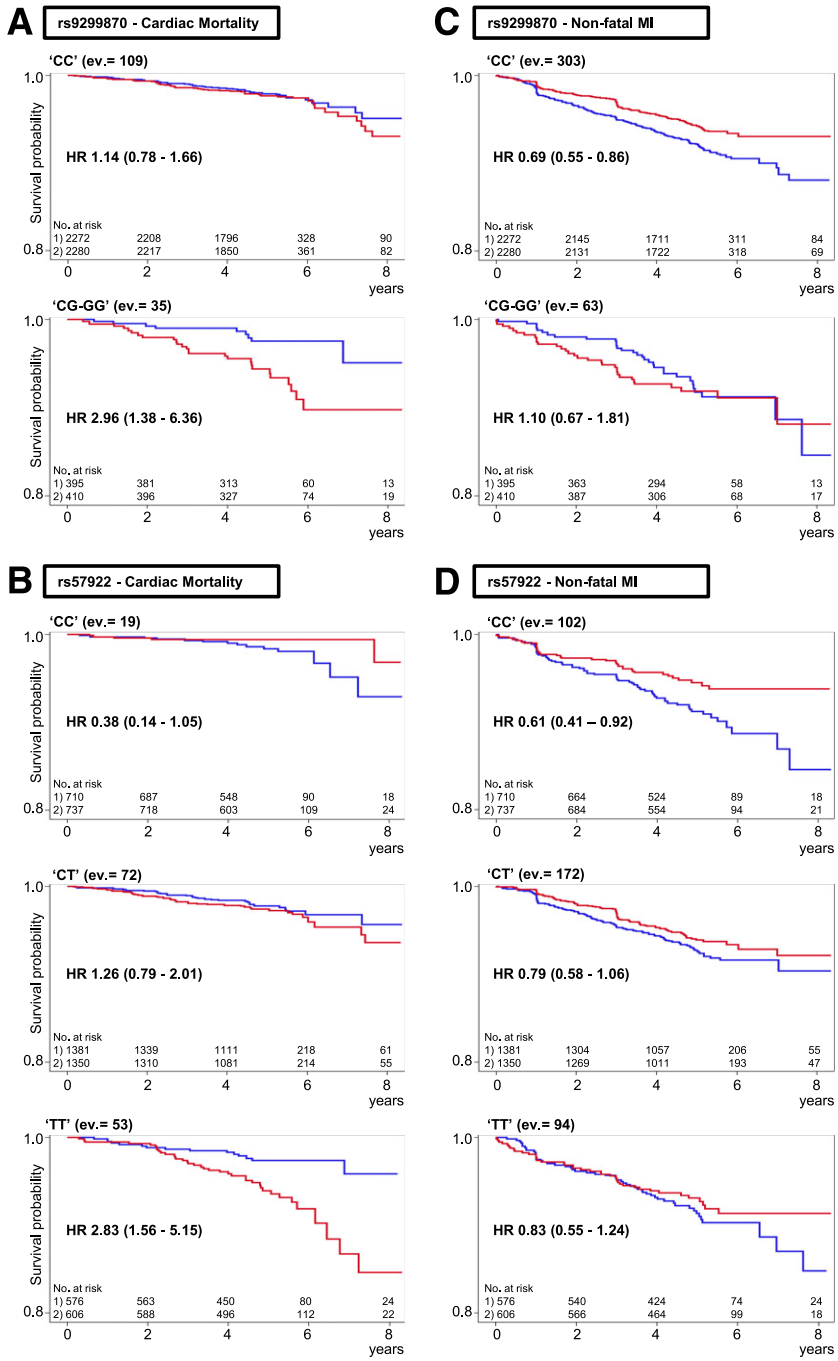

Figure 2—Influence of polymorphisms rs9299870 and rs57922 on the effect of intensive glycemic treatment on cardiovascular outcomes. Kaplan-Meier curves for cardiovascular death (*A* and *B*) and nonfatal myocardial infarction (*C* and *D*) are shown for intensive (red line) and standard (blue line) treatment after stratification by rs9299870 genotypes or by rs57922 genotypes. Homozygotes for the rs9299870 minor allele were considered together with heterozygotes because of their small number. Numbers of subjects at risk at various time points in each treatment arm are shown in the bottom panel of each plot (1 = intensive and 2 = standard). HR and 95% CI for the effect of intensive vs. standard glycemic treatment, adjusted for blood pressure and lipid subtrial assignments and interventions within these trials, clinical center networks, and the top three principal components. MI, myocardial infarction.

assignment to intensive therapy was associated with a threefold increase in cardiac mortality (HR 3.08 [95% CI 1.82–5.21]) while yielding no significant benefit on nonfatal myocardial infarction (HR 0.95 [95% CI 0.66–1.36]). The *P* values for GRS by treatment interaction were 3.0×10^{-6} for cardiovascular mortality and 0.07 for nonfatal myocardial infarction, although the *P* value for the interaction effect on cardiovascular mortality was likely biased downward by creating the GRS using two SNPs with extreme *P* values of association with cardiovascular mortality in the intensive glycemic control arm. Adjustment for previously described risk factors for excess mortality in the intensive glycemic control arm (HbA_{1c} >8.5% [69 mmol/mol], presence of neuropathy, and aspirin use at baseline) (7) did not attenuate the effect of the GRS. No significant interaction was observed within each treatment arm between HbA_{1c} levels during treatment and GRS.

To assess the potential usefulness of the GRS to select candidates for intensive glycemic control, we used these preliminary, yet to be validated findings to estimate the possible impact of this tool on the number of cardiovascular deaths and nonfatal myocardial infarctions that one could predict, based on the results above, to be prevented or caused by treating 1,000 ACCORD participants with intensive rather than standard regimen for 5 years. If applied to 1,000 subjects with GRS ≥2, intensive treatment would cause 38 cardiac deaths, while preventing only 8 nonfatal myocardial infarctions. By contrast, if applied to 1,000 ACCORD participants selected for having GRS = 1 or 0, this treatment would prevent 3 and 14 cardiac deaths, respectively, along with 21 and 30 nonfatal myocardial infarctions.

Interaction Between GRS and Long-term Glycemic Control in a Clinical Care Setting

To evaluate the modulatory effect of the GRS on the relationship between long-term glycemic control and cardiovascular mortality, we examined a cohort of 422 Joslin patients with type 2 diabetes who experienced 124 cardiovascular deaths over an average follow-up of 13 years (Supplementary Table 9). Long-term glycemic exposure was estimated from the HbA_{1c} measurements available for this cohort in the Joslin electronic medical records (median *n* = 23, IQR 12–37) over a median time period of 10 years (IQR 6–16). In this cohort, good glycemic control (defined as an average HbA_{1c} in the lower quartile of the distribution [<7.5% (58 mmol/mol)] and considered as a time-dependent variable) was overall associated with a protective effect on cardiovascular mortality

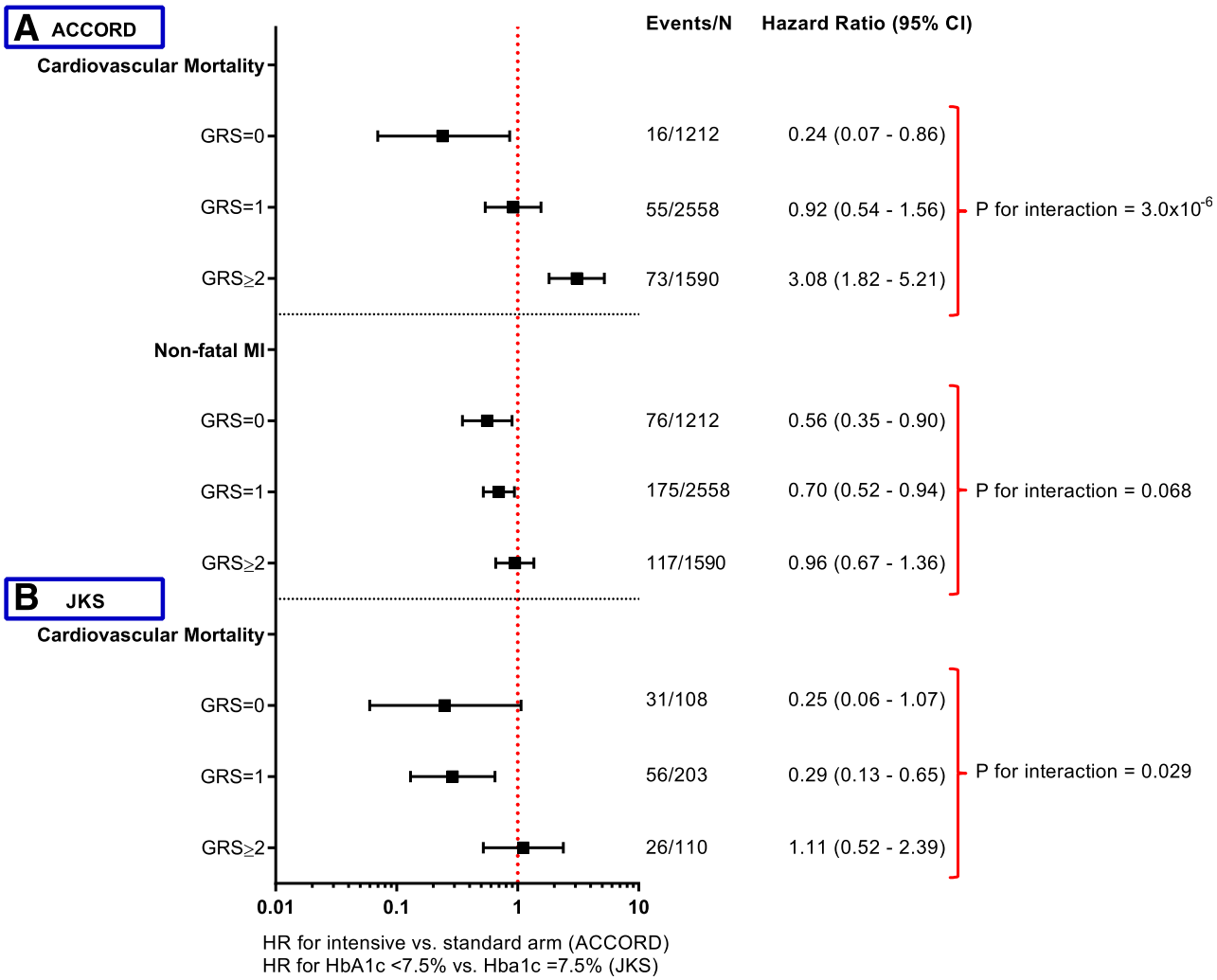

Figure 3—Influence of the GRS on the effect of glycemic control on cardiovascular outcomes in ACCORD and JKS cohorts. *A*: Forest plots for cardiovascular death and nonfatal myocardial infarction in ACCORD, depicting effects of intensive vs. standard glycemic treatment after stratification by GRS categories. GRS was obtained by adding the risk allele dosages of the top two genome-wide significant variants, rs57922 and rs9299870, giving a range of 0–4. GRS categories were then assigned as 0, 1, and ≥2 based on continuous GRS ranges of 0–0.49, 0.5–1.49, and ≥1.5, respectively (see Supplementary Fig. 9 for distribution of GRS). *B*: Effects of good vs. poor glycemic control (as per HbA_{1c} threshold below and above 7.5% [58 mmol/mol]) on cardiovascular mortality in the JKS cohort. Here, GRS categories of 0, 1, and ≥2 depict the total number of risk alleles of the two variants combined. HbA_{1c}, time-dependent covariate formulated as yearly time-weighted HbA_{1c} averages estimated from baseline up until the end of each year of follow-up. MI, myocardial infarction.

(HR 0.46 [95% 0.28–0.76], *P* = 0.002). However, when stratified by GRS, good glycemic control was associated with lower mortality among subjects with GRS = 0 or 1 (HR 0.25 [95% CI 0.06–1.07] and HR 0.29 [95% CI 0.13–0.65], respectively) but not among patients with GRS ≥2 (HR 1.11 [95% CI 0.52–2.39]) (Fig. 3*B*). The difference in the effect of glycemic control among GRS classes was statistically significant (*P* = 0.029).

Further Evidence for an Effect of the GRS on Cardiovascular Mortality

The GRS developed in ACCORD was also a significant predictor of cardiovascular mortality among participants of the ORIGIN trial, whose level of glycemic control during the study was similar to that of participants in the ACCORD intensive arm. The association was in the same direction as in that arm, with a 1-unit increase in GRS being associated with 27% higher hazards of cardiovascular death (95% CI 1.03–1.58; *P* = 0.03), and was independent of assignment to standard versus glargine arm (*P* for interaction = 0.57). No significant evidence of interaction between GRS and HbA_{1c} was observed in this study; although this analysis was limited by the fact that a vast majority of participants had postrandomization HbA_{1c} values <7.5% (58 mmol/mol).

CONCLUSIONS

Intensive glycemic control, that is, aiming for an HbA_{1c} <6.0% (42 mmol/mol) rather than between 7.0 and 7.9% (53–63 mmol/mol), significantly decreased the risk of nonfatal cardiovascular events among high-risk subjects with type 2 diabetes in the ACCORD trial (5,6). This beneficial effect, however, was offset by a paradoxical increase in mortality, mostly due to cardiovascular deaths. Through a GWAS approach, we have identified two genetic markers that were specifically associated with cardiovascular mortality in the intensive arm of ACCORD and, when considered together as a score, could

predict whether a participant in this trial was more likely to derive benefit rather than harm from the application of intensive glycemic control. Participants with the lowest score (~20% of the ACCORD population) derived on average the most benefit, experiencing a large reduction in both fatal and nonfatal cardiovascular events. Those with an intermediate score (~50% of participants) derived on average some benefit, experiencing a reduction in nonfatal events but not in cardiovascular mortality. Those with the highest scores (~30% of participants) derived on average harm, experiencing a large increase in cardiovascular deaths without any reduction in nonfatal events. The modulatory effect of these genetic markers was independent of previously identified predictors of excess mortality in the ACCORD intensive arm, such as presence of neuropathy, aspirin use, and a high HbA_{1c} at baseline (7,10).

In support of these findings, we observed a similar inverse relationship between GRS and long-term cardiovascular benefits of good glycemic control in the clinical care setting of the Joslin Clinic. The Joslin cohort, with its rich HbA_{1c} data, provided a glimpse into whether the GRS interacts with intensive glycemic control in the "real world," adding to the generalizability of our findings. Among these patients, a high GRS was associated with a neutral effect of good glycemic control rather than a detrimental one, due perhaps to the fact that this cohort was not exposed to a glucose-lowering intervention as intense as in ACCORD. It is remarkable, however, that despite the differences in design and setting, similar patterns of interaction with GRS were observed in the two studies.

Although there are no other randomized controlled trials having the same exact design as ACCORD, we were able to corroborate our findings in another, albeit different, randomized controlled trial, the ORIGIN study. ORIGIN investigated whether good glycemic control obtained by means of insulin therapy was more beneficial on cardiovascular outcomes than glycemic control obtained by other means (14). We found that the GRS was a significant predictor of cardiovascular events also in this study, regardless of the type of treatment. Since both arms were on average in excellent glycemic control at baseline (median HbA_{1c} 6.4% [46 mmol/mol] in both arms) as well as during the intervention (median HbA_{1c} 6.0–6.5% [42–48 mmol/mol]) (14), these results are consistent with findings in ACCORD and the JKS, where associations between GRS and cardiovascular mortality were only found in the presence of good glycemic control and/or intensive treatment.

These findings have potential implications for the treatment of patients with type 2 diabetes. After the report of increased mortality in response to intensive glycemic control in ACCORD, this intervention was dismissed as a viable strategy to decrease cardiovascular risk in high-risk patients with type 2 diabetes. The results of our study suggest that it may be possible to revive this therapeutic approach by developing a precision medicine strategy (21), through which intensive treatment is prescribed for those patients who will benefit from it and who are at lower risk of being harmed. The fact that testing for two genetic markers is inexpensive and can be conducted at any point in time makes this possibility especially attractive, although the cost-effectiveness of this approach will have to be evaluated. However, before this possibility can be entertained, these findings must be replicated by other studies. Also, one must consider that ACCORD was specifically directed to subjects with type 2 diabetes at high cardiovascular risk (12) and the genome-wide study was limited to those participants who consented to genetic studies (80% of the total) and self-identified as whites. Whether the described genetic effects also concern subjects with diabetes with different characteristics remains to be determined.

In addition to their potential as predictive tools, the two variants that we have identified could provide new insights into the mechanisms through which intensive glycemic control affects cardiovascular outcomes, although these can only be speculative at this time. The variant on chromosome 10 (rs9299870) is placed in intron 1 of the *MGMT* gene and associated with tissue expression of this gene as per our analysis of GTEx data. In addition to being involved in DNA repair, MGMT functions as a negative regulator of ESR1 (estrogen receptor 1) (22), which has been linked, although not unequivocally, to atherosclerosis and thrombosis (23,24). A search of the RegulomeDB database (25) shows robust evidence for a regulatory function of rs9299870 based on its occurrence on a DNAse I hypersensitivity cluster where it affects the binding of the transcriptional coactivator CREBBP. As this protein has also been implicated in the increased atherogenesis of diabetes (26), our findings may point to an as yet undescribed CREBBP-MGMT-ESR1 pathway linking glucose metabolism to cardiovascular outcomes. The other variant (rs57922) is placed in an intergenic region and associated with *NSA2* expression. Interestingly, *NSA2* is a hyperglycemia-induced gene associated with diabetic nephropathy and involved in the TGF-β1 pathway (27,28). Also, close to rs57922 is a cluster of three LINC RNAs. LINCs are thought to have important regulatory functions, affecting gene expression and cellular processes (29,30), and have been implicated in the pathogenesis of cardiovascular disease, including vascular complications of diabetes (31,32).

Our genome-wide screen also identified 22 other loci that did not reach genome-wide significance but had *P* values $<10^{-5}$ for association with cardiovascular mortality in the ACCORD intensive arm. Of these, the MASP2 protein interacts with another CAD-related gene (*MLB2*) (33), whereas the platelet phospho-fructokinase (*PFKP*) gene is linked to BMI and interacts with obesity gene *FTO* (34).

Strengths of our study include the randomized design, rigorous clinical trial protocol for the ACCORD cohort, and the availability of rich phenotype data with frequent follow-up and high rates of adherence (6,12). Another important strength is the genome-wide approach, allowing the systematic search for genetic effects without preconceived a priori hypotheses. This was further enhanced by the use of an enriched variant set with wide coverage and excellent imputation quality, and by the application of stringent criteria to evaluate significance. Overall, since this analysis tested the effect of genes on clinical cardiovascular outcomes in a cohort enrolled and monitored under rigorous clinical trial conditions, one could anticipate a strong possibility of uncovering novel associations that would be missed or

diluted in typical heterogeneous cross-sectional GWAS, even large ones.

Nonetheless, some limitations should be acknowledged. In addition to the need for replication and the uncertain generalizability mentioned above, one should consider that, due to the relatively small number of events and the stringent significance threshold, the study was powered to detect only large genetic effects. We cannot exclude the existence of other variants exerting a smaller but still relevant influence on the cardiovascular effects of intensive glycemic control. Similarly, one cannot exclude additional genetic influences by low-frequency variants, which were not included in the present analysis. Finally, although our GWAS identified two loci with robust statistical associations with cardiovascular mortality in the intensive glycemic arm of ACCORD, the tests for interaction between treatment and these two loci (singly or combined in a GRS) likely provided downwardly biased *P* values, due to a form of "winner's curse." The test of gene-treatment interaction in the Joslin cohort is not biased, however, and the results from this study suggest that the observed interaction is not solely due to statistical artifact.

In summary, we have identified two genome-wide significant loci associated with increased risk of cardiovascular death in the intensive glycemic treatment arm of ACCORD. Our additional analyses suggest that these loci could be potentially used as screening tools to identify subjects with type 2 diabetes who may highly benefit from intensive glycemic control rather than derive harm from it, although further validation is needed. These two loci also point to novel candidate pathways linking glycemic control to cardiovascular outcomes, the study of which may lead to the development of new interventions to prevent cardiovascular disease in diabetes.

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Author Contributions. H.S.S., J.C.M., and A.D. designed the study; acquired, analyzed, and interpreted the data; and wrote the manuscript. H.G. acquired and interpreted the data and reviewed the manuscript. M.L.M. analyzed and interpreted the data and wrote and reviewed the manuscript. J.S. and P.B. analyzed and interpreted the data and reviewed the manuscript. S.M. acquired the data, wrote part of the Supplementary Methods, and reviewed the manuscript. G.P. acquired, analyzed, and interpreted the data and reviewed the manuscript. G.C.M. and C.M. acquired and analyzed the data and reviewed the manuscript. T.H., S.M.M., M.J.W., A.A.M.-R., and J.B.B. acquired the data and reviewed the manuscript. R.J.S. designed the study and reviewed the manuscript. H.C.G. designed the study, acquired and interpreted the data, and reviewed the manuscript. P.K. designed the study, interpreted the data, and reviewed the manuscript. A.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix

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