Modulation of GLP-1 Levels by a Genetic Variant That Regulates the Cardiovascular Effects of Intensive Glycemic Control in ACCORD

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OBJECTIVE

A genome-wide association study in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial identified two markers (rs57922 and rs9299870) that were significantly associated with cardiovascular mortality during intensive glycemic control and could potentially be used, when combined into a genetic risk score (GRS), to identify patients with diabetes likely to derive benefit from intensive control rather than harm. The aim of this study was to gain insights into the pathways involved in the modulatory effect of these variants.

RESEARCH DESIGN AND METHODS

Fasting levels of 65 biomarkers were measured at baseline and at 12 months of follow-up in the ACCORD-Memory in Diabetes (ACCORD-MIND) MRI substudy ($n = 562$). Using linear regression models, we tested the association of the GRS with baseline and 12-month biomarker levels, and with their difference (Δ), among white subjects, with genotype data ($n = 351$) stratified by intervention arm.

RESULTS

A significant association was observed between GRS and ΔGLP-1 (glucagon-like peptide 1, active) in the intensive arm ($P = 3 \times 10^{-4}$). This effect was driven by rs57922 ($P = 5 \times 10^{-4}$). C/C homozygotes, who had been found to derive cardiovascular benefits from intensive treatment, showed a 22% increase in GLP-1 levels during follow-up. By contrast, T/T homozygotes, who had been found to experience increased cardiac mortality with intensive treatment, showed a 28% reduction in GLP-1 levels. No association between ΔGLP-1 and GRS or rs57922 was observed in the standard treatment arm.

CONCLUSIONS

Differences in GLP-1 axis activation may mediate the modulatory effect of variant rs57922 on the cardiovascular response to intensive glycemic control. These findings highlight the importance of GLP-1 as a cardioprotective factor.

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With the global rise in type 2 diabetes, it is imperative to prevent its cardiovascular complications, since these are major contributors to the high mortality, morbidity, and socioeconomic burden associated with this disease (1). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) randomized clinical trial aimed to study whether intensive as opposed to standard glycemic control could prevent cardiovascular disease (CVD) in type 2 diabetes (2). Despite a significant reduction in nonfatal myocardial

infarctions in subjects treated intensively, the trial was prematurely terminated owing to a paradoxical increase in cardiovascular mortality in the same arm (2).

Through a genome-wide association study (GWAS) on a subset of ACCORD participants, we have recently identified two genetic markers, rs9299870 on 10q26 and rs57922 on 5q13, that were associated with the excess cardiovascular mortality in the intensive glycemic arm (3). A genetic risk score (GRS) derived from these two variants significantly modulated the effect of glycemic treatment on cardiovascular outcomes. Intensive glycemic control prevented fatal and nonfatal cardiac events among subjects with a GRS = 0, whereas it increased cardiac mortality among those with a GRS $\geq$2 (3).

While the rs57922/rs9299870 GRS is a potential tool for precision medicine approaches to treat type 2 diabetes, regardless of the mechanism of its effect, understanding the pathways that are involved in this genetic modulation may provide new insights into the links between glycemic control and cardiovascular outcomes. To this end, we examined the association between the GRS and 65 biomarkers measured at baseline and at 12 months postrandomization as part of the ACCORD-Memory in Diabetes (ACCORD-MIND) MRI substudy (4). Our findings point to an unexpected link between one of the two GRS variants (rs57922) and the GLP-1 axis.

RESEARCH DESIGN AND METHODS

Study Population
In ACCORD, 10,251 individuals with type 2 diabetes and high cardiovascular risk from 77 clinical centers across the U.S. and Canada were randomized in a 1:1 ratio to intensive (HbA$_{1c}$ <6.0% [42 mmol/mol]) and standard (HbA$_{1c}$ 7–7.9% [53–63 mmol/mol]) glycemic treatment, as well as to lipid and blood pressure subtrials, in a double 2 × 2 factorial design (2). GWAS data were generated for 8,084 participants, 5,360 of whom were self-reported whites (3). From the overall ACCORD cohort, 2,977 participants were enrolled in the ACCORD-MIND study, and 632 of these underwent MRI scans (4). ACCORD-MIND MRI subjects with available serum samples participated in an ancillary biomarker study (n = 562) (4). This subset did not differ significantly from the overall cohort, except for a

larger proportion of whites, females, and blood pressure subtrial participants and lower numbers with baseline CVD (Supplementary Table 1). The current study concerned 351 subjects corresponding to the overlap between ACCORD whites with GWAS data (n = 5,360) and the ACCORD-MIND MRI biomarker cohort (n = 562).

Genotyping
Genotyping and quality-control methods of the ACCORD-genetic data set have previously been described (3). Briefly, genotyping was carried out on two platforms at two sites based on the level of genetic consent. Those participants who had consented to genetic studies by any investigator were typed on an Illumina HumanOmniExpressExome-8, version 1.0, chip at the University of Virginia, and those who had consented only to studies by ACCORD investigators were genotyped on an Affymetrix Axiom-Biobank1 chip at the University of North Carolina (3). Two distinct sets emerged after various merge and quality-control procedures: one including 5,971 samples with any level of genetic consent (ANYSET), genotyped for 1,263,585 variants, and another one including 2,113 samples with only ACCORD investigator consent (ACCSET), genotyped for 572,192 variants. High-quality imputation performed by IMPUTE version 2.3.1 (Marchini Laboratory, Oxford) on both sets resulted in 24 million variants spanning the entire genome, of which 6.8 million common (minor allele frequency $\geq$5%) single nucleotide polymorphisms (SNPs) were tested in the GWAS (3). For the current study, posterior genotype probabilities of rs57922 and rs9299870 were extracted from the GWAS data.

Biomarker Measurement
Fasting blood samples were collected at baseline and at 12 months of follow-up (4). Serum sample aliquots (1 mL) were stored at −80°C at the ACCORD central laboratory (Northwest Lipid Metabolism and Diabetes Research Laboratories), and levels of 65 biomarkers were measured immediately on freshly thawed samples (4).

Apolipoproteins A-I and B, hs-CRP, and cystatin C were measured on a Siemens BNII nephelometer (Siemens Healthcare Diagnostics, Newark, DE) using Siemens reagents and in-house prepared calibrators and quality-control materials. The interassay coefficients of variation (CVs)

of the quality-control samples with low, medium, and high levels were consistently <3% for all four biomarkers.

Nonesterified fatty acids were measured using Wako Diagnostics reagents (Wako Diagnostics, Richmond, VA) on a Roche Modular P autoanalyzer. The sensitivity of the method was 0.0014 mEq/L, and the linearity was 4.0 mEq/L. The interassay CVs, as determined on quality-control samples with low, medium, and high levels of nonesterified fatty acids, were 4.6%, 3.3%, and 3.3% respectively.

Total testosterone levels were determined by a Tosoh 2000 autoanalyzer (Tosoh Bioscience, Inc., San Francisco, CA). The assay sensitivity was 10 ng/dL. The interassay CVs on quality-control samples with high, medium, and low levels of testosterone were 2.48%, 2.69%, and 5.99%, respectively.

Estradiol (E-2) levels were measured by a Tosoh AIA-2000 analyzer in a competitive enzyme immunoassay. The assay was linear up to 3,000 pg/mL, and the sensitivity was 25 pg/mL. The interassay CVs on quality-control samples with high, medium, and low levels of estradiol were 4.2%, 4.9%, and 8.9%, respectively.

C-peptide levels were determined by a two-site immunoenzymometric assay using a Tosoh 2000 autoanalyzer calibrated against the World Health Organization's International Standard of 84/510. The assay had a sensitivity level of 0.05 ng/mL. The interassay CVs for low, medium, and high C-peptide control samples were 3.2%, 1.6%, and 1.8%, respectively.

Analyses for GAD-65 autoantibodies were based on a protocol provided by the National Institute of Diabetes and Digestive and Kidney Diseases Autoantibody Harmonization Committee. Based on the analysis of 550 samples provided by the Centers for Disease Control and Prevention Diabetes Autoantibody Standardization Program, positive/negative cutoff for the assay was determined to be 33 DK units. The GAD assay had 76% sensitivity and 92.2% specificity.

Analysis of glycated albumin was performed by means of the bromocresol purple method using a Lucica GA-L kit (Asahi Kasei Pharma, Tokyo, Japan) on a Roche Modular P autoanalyzer. The assay linear range was 3.2–68.1%, and the interassay CV was 3.0%.

The remaining biomarkers were measured by multiplexing kits from Millipore and Luminex using a single lot of reagent and

quality-control materials. These assays were grouped into the following panels.

1. Chemokine/cytokines: interleukins (IL-1α, IL-1β, IL-1ra, IL-5, IL-6, IL-8, IL-10, IL-12[p40], IL-12[p70], IL-15, and IL-17), epidermal growth factor (EGF), granulocyte-colony stimulating factor (G-CSF), interferon-γ (IFNγ), interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1(MCP-1), macrophage inflammatory proteins (MIP-1α and MIP-1β), soluble CD40 L (sCD40 L), transforming growth factor α (TGFα), tumor necrosis factor α (TNFα), vascular endothelial growth factor (VEGF), rotaxin, and fractalkine.

2. Endocrine: adiponectin, plasminogen activator inhibitor-1 (PAI-1) (active and total), resistin, hepatocyte growth factor (HGF), leptin, and nerve growth factor (NGF).

3. Metabolism: amylin (active and total), glucagon, gastric inhibitory polypeptide (GIP), GLP-1 (active), ghrelin (active), pancreatic polypeptide, and peptide YY (PYY).

4. CVD: matrix metallopeptidase-9 (MMP-9), myeloperoxidase (MPO), soluble E-selectin, soluble adhesion molecules sICAM and sVCAM, serum amyloid A, serum amyloid P, and N-terminal pro–b-type natriuretic peptide (NT-proBNP).

5. Sepsis/apoptosis: macrophage migration inhibitory factor (MIF), sFas, and sFasL.

6. Bone: osteoprotegrin (OPG) and osteocalcin.

Statistical Analysis

All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC). Baseline characteristics of participants were described through means, medians, and proportions as appropriate. Minor allele dosages (ranging 0–2) for each of the polymorphisms, rs9299870 and rs57922, were calculated from the imputed posterior genotype probabilities for each individual, and these dosages were added up to form the GRS (scoring 0–4). Biomarkers with values above and below the limits of quantification were assigned the upper and lower limits of levels of quantification, respectively. The 65 biomarkers were log transformed for normalization of their distributions, and Δ values, were obtained by subtracting baseline from 12-month log-transformed levels.

Linear regression models tested the association of the GRS in each treatment arm with baseline and 12-month biomarker levels and their difference (Δ), adjusted by blood pressure and lipid subtrials, clinical center network, and genotyping set (ACCSET or ANYSET). In a principal component analysis including 65 biomarker measurements at baseline, 65 measurements at 12 months, and 65 Δ values, 100% of the variance of these 195 variables (65 × 3) was explained by 70 principal components. On this basis, a Bonferroni-adjusted P value threshold of 3.6×10^{-4} (0.05/[70 × 2 treatment arms]) was set as a significance threshold for our primary analysis. As the effect of GRS was significant for ΔGLP-1, the modulatory effects of polymorphisms and treatment on GLP-1 levels were evaluated by estimating least squares (LS) means of baseline, 12-month, and ΔGLP-1 levels within genotype and treatment groups. Further sensitivity analyses included adjustments for baseline CVD, age, sex, HbA_{1c}, fasting plasma glucose, heart rate, BMI, and glomerular filtration rate (GFR).

The associations between SNP rs57922 and the expression of selected genes suggested by the literature to be involved in GLP-1 synthesis or secretion (5–9) were tested using RNA-Seq data from up to 570 donors in the online Genotype-Tissue Expression (GTEx) database v6p (The Broad Institute of MIT and Harvard, Cambridge, MA). This publicly available database allows the identification of potential expression quantitative trait loci (eQTLs), that is, genetic variants affecting gene expression in different tissues. Random effects meta-analyses of rs57922 eQTLs across multiple tissues were conducted for each gene using Metasoft, version 2.0.1 (10).

RESULTS

Baseline characteristics of the ACCORD participants included in this study did not significantly differ between intensive and standard glycemic arms (Table 1). Supplementary Table 2 shows the results of the analysis for association between the rs57922/rs9299870 GRS, which had been found to be associated with cardiovascular mortality in the intensive arm, and 65 biomarkers measured at baseline and 12 months after randomization. No significant association was observed for any of the biomarkers at either time point in either treatment arm. However, when

biomarkers data were expressed as the difference (Δ) between 12-month and baseline levels, a highly significant association was observed in the intensive treatment arm for active GLP-1, with each GRS unit increment being associated with a 22% decrease in the change in active GLP-1 levels from baseline to 12 months ($P = 3 \times 10^{-4}$) (Table 2). No association was observed in the standard arm, resulting into a GRS × treatment interaction P value of 0.016 (Table 2).

As shown in Figs. 1 and 2, the GRS effect on active GLP-1 levels was mostly driven by SNP rs57922. No significant differences were observed among rs57922 genotypes in active GLP-1 levels at baseline in either treatment arm (Fig. 1A). In the intensive arm, active GLP-1 levels increased from baseline to 12 months by 22% (95% CI 1–46) among C/C homozygotes, were unmodified in C/T heterozygotes, and decreased by 28% (95% CI 10–41) in T/T homozygotes ($P = 5 \times 10^{-4}$) (Fig. 2A). By contrast, no significant changes in active GLP-1 levels from baseline to 12 months were observed in the standard treatment arm for any of the genotype groups. Carriers of the rs9299870 minor (G) allele showed an overall tendency to have higher active GLP-1 at baseline (Fig. 1B), but no significant differences were observed in the change from baseline to 12 months (Fig. 2B) in any of the treatment/genotype groups. Adjustments for baseline CVD, diabetes duration, HbA_{1c}, fasting plasma glucose, heart rate, smoking, age, and sex did not alter these results (Supplementary Table 3). No significant correlations were observed between ΔGLP-1 levels and ΔHbA_{1c} ($r^2 = 0.01$) or between ΔGLP-1 and Δfasting plasma glucose ($r^2 = 0.02$) within rs57922 genotypes in the intensive arm. In subgroup analyses, the association between active GLP-1 level changes and rs57922 genotype observed in the intensive arm was most pronounced among males ≥ 61 years and among individuals with baseline BMI <35 kg/m^2 or BMI change in the upper tertile (Supplementary Tables 4 and 5).

There were only nine subjects on the GLP-1 analog exenatide during the 12 months of follow-up and none on dipeptidyl peptidase-4 (DPP-IV) inhibitors. Repeating the analysis by excluding the nine subjects on exenatide did not alter results. With regard to other antihyperglycemic treatment modalities, no statistical

Table 1—Baseline characteristics of white participants of the ACCORD-MIND MRI biomarker cohort (N = 351) within intensive and standard glycemic arms

Characteristic*	Intensive arm (N = 162)	Standard arm (N = 189)
Female sex, n (%)	64 (39.5)	84 (44.4)
Age, years, mean (SD)	62.3 (5.7)	63.3 (5.8)
Diabetes duration, years, median (IQR)	8.8 (5.0–11.0)	9.7 (5.0–13.0)
Previous cardiovascular event, n (%)	44 (27.2)	49 (25.9)
Current smoker, n (%)	16 (9.9)	22 (11.6)
HbA$_{1c}$, %		
Mean (SD)	8.2 (1.0)	8.0 (0.9)
Median (IQR)	8.0 (7.6–8.7)	7.9 (7.5–8.5)
Fasting serum glucose, mg/dL, mean (SD)	179.4 (52.6)	174.6 (46.5)
BMI, kg/m^2, mean (SD)	33.9 (4.7)	32.8 (4.7)
Waist circumference, cm, mean (SD)	111.2 (12.7)	107.9 (12.3)
Blood pressure, mmHg, mean (SD)		
Systolic	134.2 (16.3)	134.8 (17.4)
Diastolic	74.7 (9.5)	73.7 (9.7)
Serum creatinine, mg/dL, mean (SD)	0.9 (0.2)	0.9 (0.2)
eGFR (from MDRD), mL/min/1.73 m^2, mean (SD)	86.3 (20.0)	87.5 (19.6)
Lipids, mg/dL, mean (SD)		
Total cholesterol	182.7 (38.3)	184.9 (42.7)
LDL	100.0 (31.3)	101.4 (33.4)
HDL (women)	47.3 (12.1)	47.2 (11.4)
HDL (men)	38.1 (9.1)	39.9 (9.1)
Triglycerides	214.7 (129.7)	208.2 (124.2)
Blood pressure trial, n (%)	102 (63.0)	116 (61.4)
Standard	48 (29.6)	51 (26.9)
Intensive	54 (33.3)	65 (34.4)
Lipid trial, n (%)	60 (37.0)	73 (38.6)
Statin + placebo	33 (20.4)	31 (16.4)
Statin + fibrate	27 (16.7)	42 (22.2)

eGFR, estimated glomerular filtration rate; IQR, interquartile range. *There were no significant ($P < 0.05$) differences between the two groups.

differences were observed in drug distributions across genotypes (Supplementary Table 6). Accordingly, adjustment for the different modalities yielded no or minimal attenuations of the effect of the SNP on GLP-1 change (Supplementary Table 7). Also, there was no evidence of interaction between the SNP and any of the treatment modalities (data not shown).

In a meta-analysis of multitissue eQTL data in GTEx, rs57922 was associated (random effects P values <0.05) with the expression of four out of twenty genes selected for their involvement in the synthesis, secretion, and/or processing of GLP-1 (binomial $P = 0.01$) (Supplementary Table 8). Significant genes included *LEPR* (leptin receptor) ($P = 0.0007$), *GRPR* (gastrin-releasing peptide receptor) ($P = 0.0009$),

SLC5A1 (sodium/glucose cotransporter 1) ($P = 0.0039$), and *SLC2A5* (glucose transporter 5) ($P = 0.03$). In all four cases, the rs57922 T allele, which in ACCORD was associated with a decrease in GLP-1 levels during intensive treatment, was associated with lower expression levels of these genes within the terminal ileum and other tissues, with no evidence of significant heterogeneity across tissues. *cis*-eQTL analyses in intestinal tissue for genes located 2 MB upstream and downstream of rs57922 did not reveal any significant association with the expression of transcription factors that could explain the *trans* effects on LEPR, GRPR, or GLUTs (data not shown). However, specific expression of these genes within intestinal L cells could not be examined owing to lack of this data in the GTEx database.

CONCLUSIONS

Intensive glycemic control has been proposed as a strategy for preventing CVD in type 2 diabetes. However, this approach has remained controversial owing to the small overall benefit of this intervention and the suspicion, raised by ACCORD, that it may cause an increased risk of cardiovascular death. We recently identified two genetic variants that can potentially be used to identify a subset of subjects with type 2 diabetes who may derive greater benefit from intensive glycemic control without being exposed to its risks (3). Following up on those findings, we have now discovered a possible pathway that could mediate the modulatory effect of one of those variants (rs57922). Specifically, we have found that homozygotes for allele C of these variants, that is, those individuals who derived benefit from intensive glycemic control, responded to this intervention with an increase in fasting levels of active GLP-1 at 12 months from randomization. By contrast, T/T homozygotes, that is, those subjects who showed increased cardiovascular mortality in response to intensive glycemic control, were characterized by a significant reduction in 12-month active GLP-1 levels. While in the limited sample at our disposal we could not test the association of GLP-1 changes with cardiovascular mortality, one can hypothesize that the observed difference in GLP-1 levels may have contributed to the stark difference between the two genotype groups in the cardiovascular response to intensive glycemic control.

Table 2—Association of GRS with baseline and 12-month GLP-1 levels and with ΔGLP-1 levels in ACCORD intensive and standard arms

	Intensive glycemic arm		Standard glycemic arm		
	Fold change (95% CI)*	P	Fold change (95% CI)*	P	GRS × treatment interaction P
Baseline GLP-1	1.15 (0.93–1.42)	0.193	1.03 (0.84–1.25)	0.810	0.457
12-month GLP-1	0.86 (0.70–1.06)	0.161	1.03 (0.85–1.24)	0.778	0.227
ΔGLP-1	0.78 (0.68–0.89)	0.0003‡	0.99 (0.86–1.15)	0.935	0.016

*Fold change of GLP-1 levels per unit of GRS obtained from linear regression models testing association of GRS with log-transformed active GLP-1 levels at baseline and 12 months, and the difference between the two, adjusted by clinical center networks and source of genetic data (ACCSET or ANYSET). ‡Significant at $P < 0.00036$ (Bonferroni adjusted).
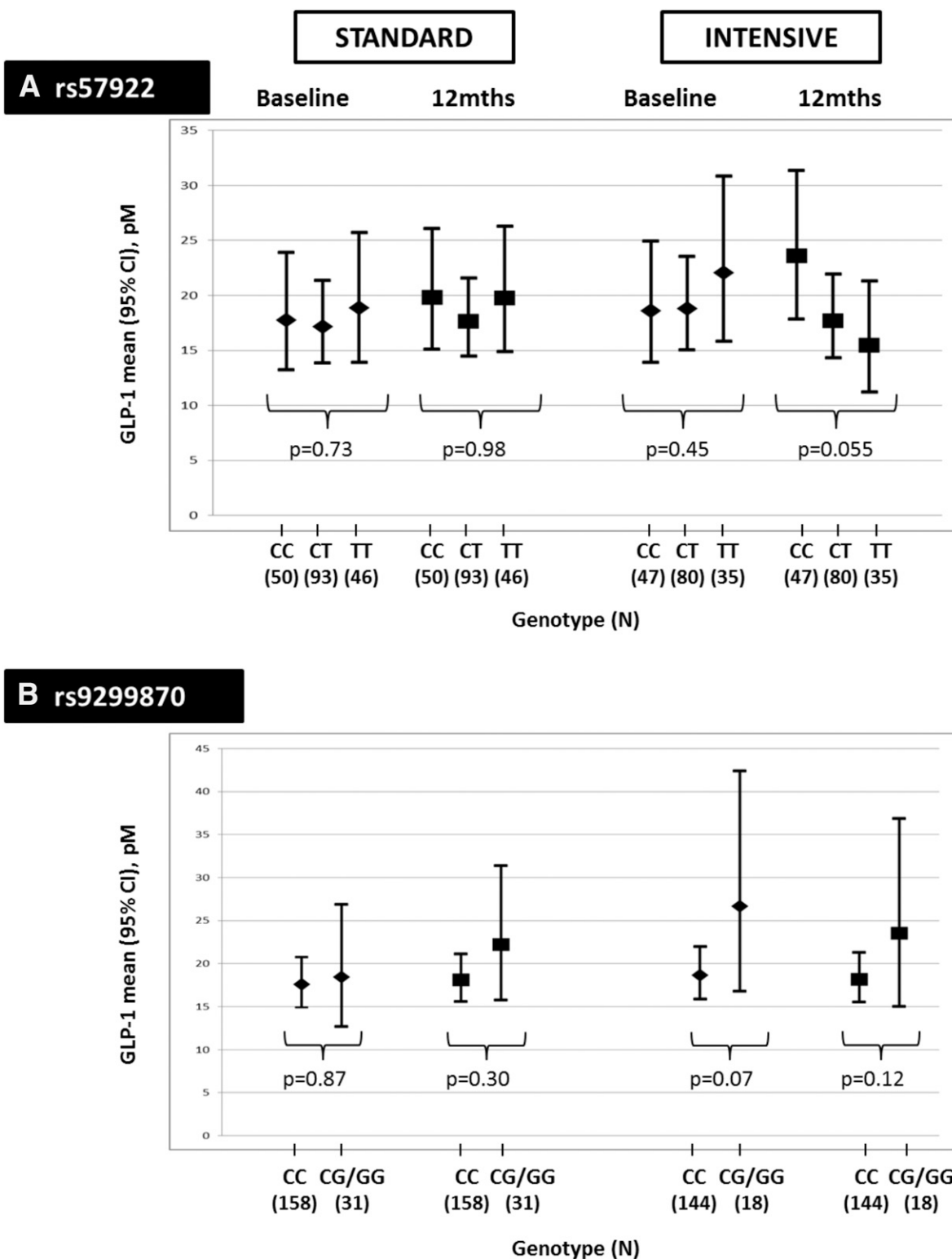
Figure 1—*A* and *B*: Baseline and 12-month GLP-1 levels within glycemic treatment arms and genotypes of rs57922 and rs9299870. LS means of GLP-1 obtained from model adjusted by trial covariates, clinical center network, and source of genetic data. *P* values are obtained from generalized linear regression for association with baseline or 12-month GLP-1 levels, using additive model of SNP, and adjusted for trial covariates, clinical center network, and source of genetic data. 12mths, 12 months.

How the interaction of intensive glycemic control and the rs57922 variant may affect active GLP-1 plasma levels is unclear at this time. GLP-1 is secreted by intestinal L cells after being cleaved from a precursor (preproglucagon) by the action of the enzyme prohormone convertase 1. Once secreted, active GLP-1 is quickly inactivated by cleavage of its NH_2 terminus by the enzyme DPP-IV. SNP rs57922 is not placed in or within the vicinity of the genes coding for

A rs57922

12mth/baseline GLP-1 ratio (95% CI)

STANDARD
- CC   1.12 (0.91-1.38)
- CT   1.02 (0.88-1.19)   p=0.68
- TT   1.05 (0.85-1.31)

INTENSIVE
- CC   1.22 (1.01-1.46)
- CT   0.95 (0.82-1.09)   p=5x10^{-4}
- TT   0.72 (0.59-0.90)

B rs9299870

STANDARD
- CC   1.03 (0.92-1.16)
- CG/GG   1.17 (0.90-1.53)   p=0.37

INTENSIVE
- CC   0.97 (0.88-1.08)
- CG/GG   0.88 (0.65-1.19)   p=0.67
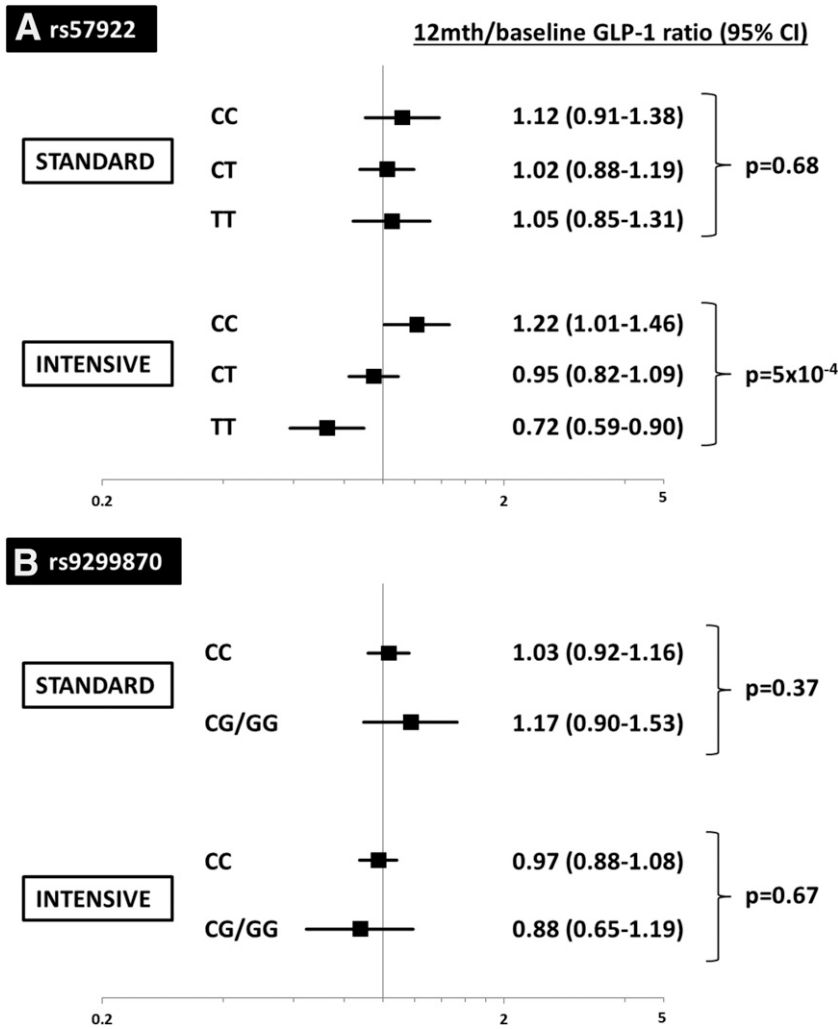
Figure 2—*A* and *B*: Mean change in GLP-1 from baseline to 12 months within glycemic treatment arms and rs57922 genotypes. Twelve-month–to–baseline GLP-1 ratio derived from the difference (Δ) between the log-transformed baseline and 12-month GLP-1 levels; here, presented within intensive/standard glycemic treatment arms, are LS means of this ratio (from model adjusted for trial covariates, clinical center network, and source of genetic data) within genotypes of rs57922 on 5q13 (*A*) and rs9299870 on 10q23 (*B*). *P* values are obtained from generalized linear regression for association with ΔGLP-1 levels, using additive model of SNP, and adjusted for trial covariates, clinical center network, and source of genetic data. 12mth, 12-month.

preproglucagon (*GCG*), prohormone convertase 1 (*PCSK1*), and *DPP-IV* and was not found to be associated with the expression of those three genes in any of the tissues in the GTEx database. We found, however, an association of the variant with the expression of genes coding for proteins involved in the stimulation of GLP-1 secretion by L cells such as the receptor of gastrin-releasing peptide (a GLP-1 secretagogue) and two hexose transporters (sodium/glucose cotransporter 1 and glucose transporter 5) mediating the sensing of nutrients in the intestinal lumen by L cells. The variant was also associated with expression of the gene coding for leptin receptor, to which the cytokine leptin binds, thereby inducing GLP-1 release via STAT (signal transducer and activator of transcription) phosphorylation (6,11). Since the allele associated with decreased GLP-1 levels during intensive glycemic control was associated with lower expression of these genes and vice versa, this appears to be a conceivable mechanism through which the variant may affect the GLP-1 response. Though *cis*-eQTL analyses in intestinal tissue could not explain the *trans* effects on LEPR, GRPR, or GLUTs, it should be considered that rs57922 is in close proximity to a number of long noncoding RNAs (LINCs), which, in theory, could be responsible for *trans* effects. Unfortunately, this hypothesis cannot be readily explored, as data on the expression of these long noncoding RNAs are not included in the GTEx database. Hence, whether the variant affects the expression of these genes directly through a long-range *trans* effect or indirectly by affecting other genes placed in its vicinity remains unclear at this time, as do the mechanisms of the interaction with intensive glycemic control. With regard to the latter, we did not find any difference among genotypes in the prevalence of glucose-lowering treatments that may influence GLP-1 levels such as metformin (12). None of the participants were on DPP-IV inhibitors, and only handfuls were on GLP-1 agonists during the 12 months of follow-up. We cannot exclude, however, that some drugs were used at a higher dosage in the intensive arm and that this was the feature having a permissive influence on the GLP-1 effects of the gene expression changes induced by the SNP. Unfortunately, we cannot explore this hypothesis, as we do not have access to individual level data about the dosage of each drug. Differences in HbA$_{1c}$ or fasting plasma glucose levels do not seem to account for the interaction either. Given the evidence suggesting that intestinal microbiota can regulate GLP-1 secretion (13), one can also postulate a role of differences in microbiome induced by the intensive therapy, but again we do not have data to test this hypothesis.

In terms of mechanisms though which GLP-1 levels can be related to cardiovascular outcomes, it is well-known that GLP-1, in addition to its actions on glucose metabolism, has anti-inflammatory and antioxidative effects in cell types relevant to atherogenesis as well as direct cardioprotective effects (14–16). Consistent with these findings, treatment with the GLP-1 agonists liraglutide and semaglutide have been reported to reduce the incidence of major cardiovascular events (death from cardiovascular causes, nonfatal myocardial infarction, and nonfatal stroke) in individuals with type 2 diabetes independent of the effects of these drugs on glycemic control (17–19). Furthermore, by playing a regulatory role in the hypothalamic-pituitary-adrenal axis, GLP-1 modulates neuroendocrine and autonomic responses to acute and chronic stress, which may influence the ability of a subject to survive a harmful exposure (20–22). One must consider, however, that these anti-inflammatory,

antiatherogenic, and antistress effects have been observed with pharmacological doses of native GLP-1 or GLP-1 agonists (17,18,23–25) and/or in animal models with homozygous disruption of the GLP-1 receptor (20,21,26). Whether the relatively small changes in fasting GLP-1 levels that we observed in response to intensive glycemic control in carriers of specific rs57922 genotypes are sufficient to affect cardiovascular risk remains to be determined. A report from Sweden has described an association between fasting GLP-1 levels and left ventricular diastolic function in elderly men (27), but the same group failed to demonstrate an association between low GLP-1 levels and coronary artery disease (28). Further studies considering both fasting and postprandial GLP-1 concentrations in larger populations are necessary to clarify the role of nonpharmacological levels of GLP-1 activity in the modulation of cardiovascular risk.

Our study has several strengths, including 1) the meticulous design of ACCORD and its excellent randomization, follow-up, and adherence; 2) the excellent quality of DNA samples, genotyping, and imputation and the precise measurements of a large panel of biomarkers; and 3) the availability of longitudinal biomarker measurements. However, several limitations should be acknowledged. First, the sample size was relatively small, and we cannot exclude that smaller effects on other biomarkers were present but were missed owing to limited power. Also, as a result of the small sample size, we were unable to evaluate the association of GLP-1 with cardiovascular outcomes to determine how much of the modulatory effect of rs57922 was mediated by its association with GLP-1 level during the intervention. In fact, we cannot rule out the possibility of the variant being associated with a cardiovascular benefit via another mechanism not involving the GLP-1 axis, which in turn has a secondary effect on GLP-1 levels. Second, given the clinical characteristics of the ACCORD participants, our findings may not be generalizable to younger subjects, nonwhites, or those with low cardiovascular risk. Third, since biomarker measurements were limited to baseline and 12 months of follow-up, longer-term effects on GLP-1 or other biomarker levels could not be investigated. Finally, as only fasting samples were taken, we cannot

make any inference on a possible impact of rs57922 on postprandial GLP-1 levels.

In conclusion, our results suggest a possible role of diminished GLP-1 activity as a factor for increased cardiac mortality during intensive glycemic therapy in rs57922 T/T homozygotes, possibly via impaired cardio- and/or stress-protective mechanisms or enhanced inflammatory pathways. Further studies seeking replication of this observation in other data sets and exploring the underlying mechanisms are warranted.

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